JOURNAL OF AGRICULTURAL RESEARCH

VOL. XXIX WASHINGTON, D. C., JULY 15, 1924 No. 2

BACTERIAL PUSTULE OF SOYBEAN[1]

By FREDERICK A. WOLF

Botanist, North Carolina Agricultural Experiment Station

INTRODUCTION

Diseases of the soybean, *Soja max* (L.) Piper, have for several years been the subject of investigation by the writer and his colleagues at the North Carolina Agricultural Experiment Station (*2*, *3*, *8*, *11*, *16*, *17*).[2] Among those which have been given special study is a leafspot to which the appropriate name "bacterial pustule" has been applied. This leafspot disease was first briefly described (*4*) in 1922 by Florence Hedges. In this preliminary report, Miss Hedges designated the causal organism *Bacterium phaseoli* var. *sojense* and stated that a paper covering the results of her investigations was in preparation (*6*). It might be anticipated that the present report would confirm in all essential features that of Miss Hedges. Should it contribute nothing new to the knowledge of this disease and its causal organism, nevertheless it is believed to have a definite value since the investigations have been conducted entirely independently. It is the writer's purpose, therefore, to embody in this paper a description of the disease, an account of its relation to other soybean leafspots of bacterial origin, studies on its etiology, and on the morphology and physiology of the causal organism.

HISTORY AND DISTRIBUTION

A definite knowledge of the occurrence of this disease dates from Miss Hedges's isolation, from specimens sent from Texas in 1917, of a yellow organism very closely resembling *Bacterium phaseoli* E. F. Smith. However, a bacterial leafspot of soybean which was assumed to be due to *Bacterium phaseoli* was mentioned as long ago as 1904 (*12*). The isolations upon which this report was based were made two years earlier (*13*, *p. 280*) from diseased soybeans taken from two localities, one near Charleston, S. C., and the other near Washington, D. C. The organism isolated at that time was not proved by inoculation experiments to be pathogenic, and therefore its relation to the leafspot can not now be satisfactorily determined. This problem is further complicated by the fact that other bacterial organisms have recently been found to be pathogenic to soybean. The first of these diseases to be carefully investigated was a bacteriosis (*9*) which manifests itself by the formation of lesions both on stems and pods. Those on the stems are especially characteristic, since they girdle them in a manner suggesting the blackleg disease of potatoes. The pathogen, *Bacillus lathyri* Manns and Taubenhaus, is identical with the one which causes the "streak" disease of sweet pea, *Lathyrus odoratus* L. It is a yellow organism but has very different morphological and cultural characters from the soybean organism under consideration.

As will be shown later, the appearance of bacterial pustule has little in common in any stage of development with that of bacterial blight as described by Miss Coerper (*1*) or by the writer (*16*). The causal organisms identified as *Bacterium glycineum* by the former and as *Bact. sojae* by the latter are both white and are thus easily separable from the pustule organism; but in the case of old lesions caused by *Bact. sojae* the tissues are always occupied also by a yellow, one-flagellate organism (*16*) which may be a source of confusion as to the primary cause. It might be indicated at this point that although the bacterial blight diseases caused by *Bact. glycineum* and *Bact. sojae* are very similar in appearance, the causal organisms are readily distinguishable and specifically distinct, as has been shown by certain cultural studies (*11*, *7*, *17*).

Mention has been made in several previously published accounts of bacterial diseases of soybeans in the Orient. A bacteriosis has recently been recorded (*10*) by Miura, a Japanese investigator, as occurring in Manchuria. His description of the disease accords with the appearance of

[1] Received for publication Feb. 25, 1924—issued January, 1925.

[2] Reference is made by number (italic) to "Literature cited," p. 68.

bacterial blight, and he furthermore expresses the opinion that the disease in Manchuria is the same as that found in America. He also records the occurrence of this bacteriosis in other parts of China and Japan.

In 1921 another Japanese investigator, Takimoto (*14*), published a comprehensive account of his investigations on a bacterial disease of soybeans which he had had under observation since 1914. This disease manifests itself by the formation of numerous small, angular, dark-brown leaf-spots with chlorotic intervening tissues. Lesions may also form along the veins and extend along them making dark streaks. Black, sunken areas form upon both petioles and stems. These symptoms, as he points out, are quite different from bacterial blight in America. The organism which he isolated was proved to be pathogenic not only to soybean in all stages of its growth, but also to Adzuki bean, *Phaseolus angularis* Willd. Takimoto does not assign a name to the bacterium which he had under observation, but does compare it morphologically and physiologically with published accounts of *Bacterium glycineum*, as described by Miss Coerper, *Bact. sojae*, as described by the writer, and *Pesudomonas glycineum*, as described by Nakano,[3] who isolated the organism in 1916 from collections made in Kumamoto prefecture. He concludes that it is most like *Bact. sojae*, but differs from it in the absence of a capsule, in its failure to effect a change in milk, and in its growth in the closed arm of fermentation tubes containing dextrose, saccharose, and mannite. It is doubtful whether these differences would be confirmed were both forms in the hands of one investigator; and it remains for subsequent investigation to determine the identity of the organism of Takimoto and *Bacterium sojae* and whether either or both are identical with *Pseudomonas glycineum* Nakano.[4] Certainly none of these are like the soybean bacterial pustule, as will become apparent when they are compared with the description of the appearance of this disease and with the cultural characters of the causal organism.

Bacterial pustule is known to occur in Texas, Virginia, Kansas, South Carolina, and Louisiana (*3*, *4*). It has been collected from a sufficient number of localities in North Carolina to warrant the belief that it is generally prevalent throughout the State.

APPEARANCE OF THE DISEASE

Soybean pustule has not been observed upon the stems and pods, but upon the foliage only. Soybeans in all stages of growth varying from the seedling stage to mature plants are subject to infection. The disease may appear upon the foliage in any stage of maturity, but reaches its most destructive stage of development at the time when the plants have reached their maximum vegetative growth.

The first indication of the disease is the presence of minute elevations on either or both leaf surfaces (figs. 1 and 2). These elevations themselves are light green in color. Soon a yellowish-green halo forms as a border at the base of each elevation. At this stage the lesions are easily distinguished from those produced by *Bacterium glycineum* and *Bact. sojae*, neither of which causes the formation of pustules, and each of which, especially in the early stages, causes the invaded tissues to be translucent. This is followed by the enlargement of the elevations to very prominent pustular outgrowths often extending above the leaf about twice as high as the thickness of the leaf (fig. 3). These raised portions soon collapse and, together with a portion of the surrounding invaded tissues, become brown. Ultimately, the lesions become angular to irregular reddish-brown areas which vary in size from minute specks to large irregular spots. These large areas arise by the fusion of lesions in case initial lesions are abundant. Lesions in all stages of development occur on the same leaflet. This indicates that the initial infections serve as the source of inoculum for secondary infections.

The general aspect of the pustule disease in the late stages (Pls. 1, 2) is like that produced by the rust fungi, whereas bacterial blight lesions are dark brown to brownish-black in color and tend to break out and fall away, thus making perforations or notches in the leaves.

Nakano's original paper has not been seen by the writer, nor does he have a complete reference to it.

These papers were translated by Mr. S. Yonemachu, a special student of textile manufacture at the North Carolina State College of Agriculture and Engineering. Grateful acknowledgment is herewith made of his kindness and courtesy in rendering this service.

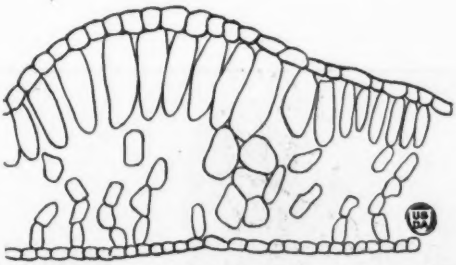


FIG. 1.—Diagram outlined with camera lucida showing beginning of pustule formation on upper leaf surface

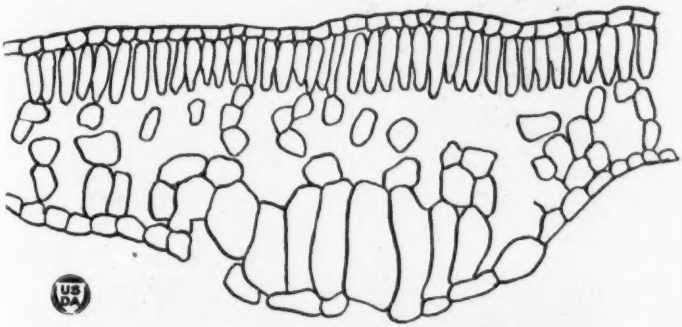


FIG. 2.—Pustule formation involving the tissues of the lower leaf surface

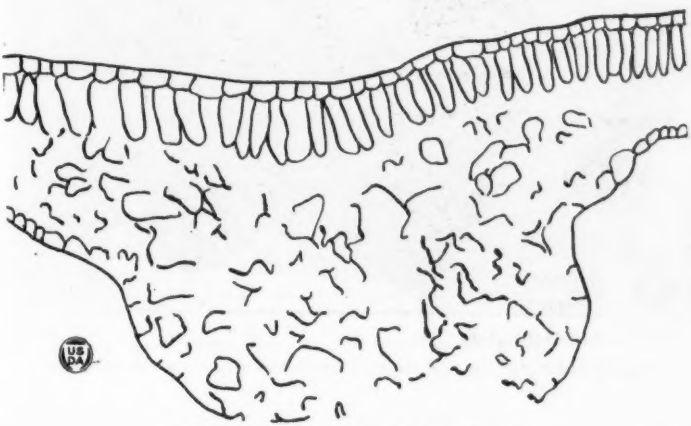
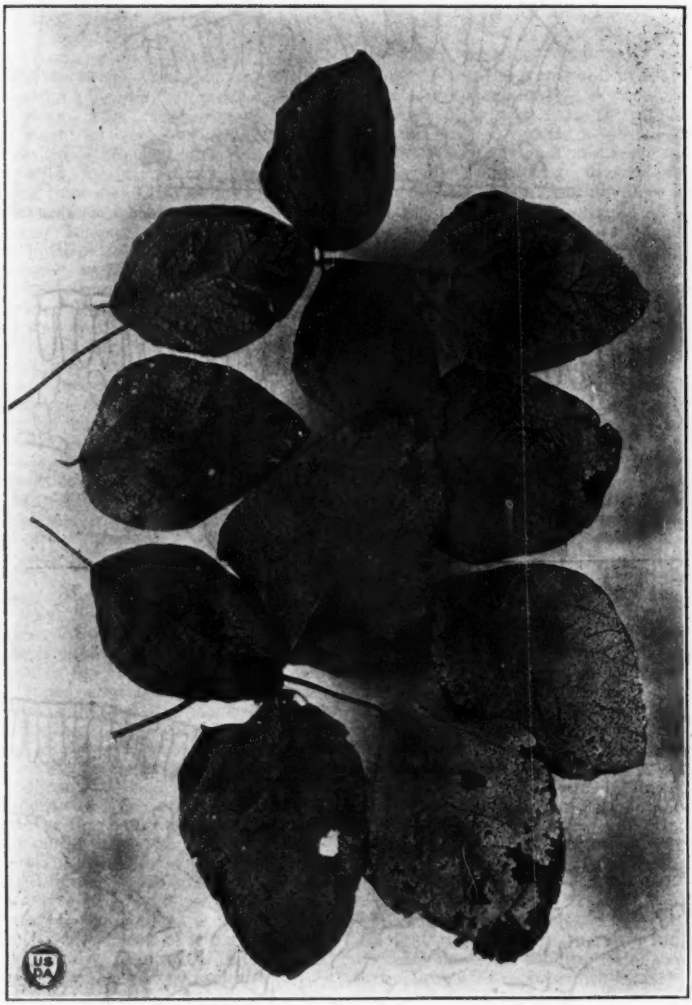
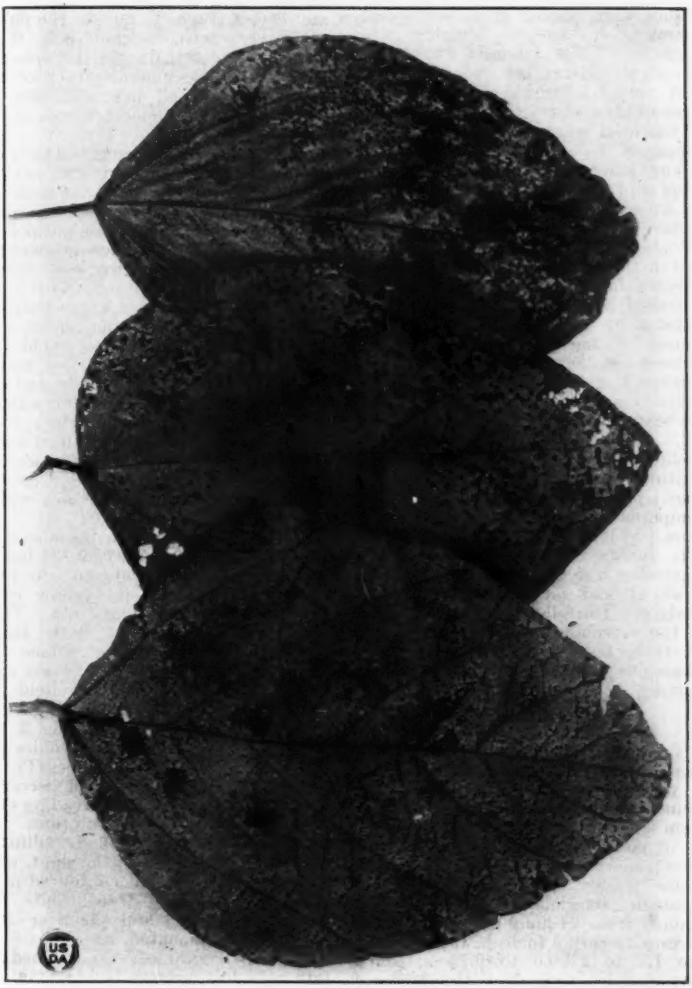


FIG. 3.—A large pustule whose cells have collapsed



Bacterial Pustule of Soybean Plate 1

Bacterial pustule of soybean showing natural infection in various stages of development.



Bacterial Pustule of Soybean Plate 2

Leaves, natural size, affected with bacterial pustule

CAUSAL ORGANISM

ISOLATION

In preparations made by macerating lesions in a drop of water, the invaded tissues were found, upon microscopic examination, to be teeming with bacteria. These bacteria swarm out in such abundance as to cloud the water and form a turbid suspension, a phenomenon characteristic of diseases of bacterial origin. If an inoculum is prepared by direct maceration of young lesions in sterile water and a loopful of this inoculum is transferred to an agar plate and spread over its surface with a zigzag stroke, discrete colonies of the pathogen form near the end of the stroke. These can then be selected for transfer to tube cultures. A considerable number of strains were isolated by this procedure during the course of the present investigation. Subsequent comparative study of these strains to determine their cultural characters and their ability to infect soybean showed them all to be identical. Since it was apparent from the preliminary studies that the soybean pustule organism is closely related to *Bact. phaseoli*, several strains were compared with *Bact. phaseoli* isolated from pod lesions of Lima bean, *Phaseolus lunatus* L., and garden bean, *Phaseolus vulgaris* L.; and with a strain of bean blight isolated by Miss Hedges. The following parallel studies of the morphology and physiology of all strains both from soybean and from Phaseolus show that all are practically identical.

MORPHOLOGY

VEGETATIVE CELLS.—The primary cause of soybean bacterial pustule is a yellow rod-shaped organism with rounded ends. When taken directly from lesions it is found to occur singly or in pairs, but tends in bouillon to form short chains. The organism stains readily with all of the more common bacteriological stains. When stained from 24-hour potato-agar cultures with carbol fuchsin, the elements are 1.3 to 2×0.6 to 0.75 μ. Such preparations, too, show the presence of investing material, as is manifest also with Welch's capsule stain; but this envelope can not be interpreted as indicating a well-defined capsule. When stained by Gram's method, the organism is decolorized. Endospores and marked involution forms have not been noted.

When the organism from young lesions, from bouillon cultures, or from 24-hour agar cultures is examined for motility, it is seen to possess the power of rather active locomotion. That this movement is due to the presence of a polar flagellum about twice the length of the cell has been demonstrated by several modifications of the method of Loeffler.

CULTURAL CHARACTERS.—The various media employed in the cultural studies were prepared by the methods employed by the writer and his associates in their studies on the physiology of certain plant pathogenic bacteria (*17*). The nutrient broths contained 1 per cent Difco peptone and 0.3 per cent Liebig's beef extract; the nutrient agars the same, with the addition of 1.8 per cent of bacto-agar. The hydrion concentration of cooled media was measured colorimetrically and the media were not heated after adjustment of reaction. The carbon compounds were sterilized in distilled water and added with aseptic precautions to cooled media. The cultures were incubated at room temperatures which approximated 20° to 25° C.

NUTRIENT AGAR.—The colonies may appear in agar plates within 24 hours, but are not prominent, nor do they show the characteristic yellow color until they are 48 hours old. They are circular in outline with entire margins and a glistening surface but with no surface markings. Their consistency varies from nonviscid to slightly viscid. In potato agar or other nutrient agar containing 3 per cent or more of agar, the colonies are convex with internal markings (Pl. 3). With media of a low degree of viscosity, the colonies are flat and spreading with no striking internal convolutions. In agar slants the growth is filiform, spreading at the base of the slant, with an entire or somewhat contoured margin, glistening and translucent. No odor is developed and the agar does not become pigmented.

POTATO CYLINDERS.—On steamed potato cylinders, growth is first manifest by a faint yellowish streak. This becomes abundant within 24 to 36 hours, is spreading, yellow, and has a striking whitish zone along the border of the growth. Within six to eight

Bacterial Pustule of Soybean Plate 3

Bacterium phaseoli var. *sojense* in plate cultures showing difference in appearance due to difference in viscosity of agar. The upper culture contains 1.8 per cent agar, the lower contains 5 per cent agar. All other conditions are identical.

days the cylinder will have collapsed, and when this collapsed tissue is tested with Lugol's solution the starch will be found to have disappeared, showing that the pathogen possesses strong diastatic properties. An ocular demonstration of the ability of the pustule-forming bacterium to hydrolyze starch may be made by growth in plate culture on beef extract agar plus 0.2 per cent of soluble starch. If the plates are flooded with iodine solution after a week's incubation, a broad clear zone surrounding the colonies will indicate the area in which the starch has been destroyed by enzym activity.

MILK.—Plain sterilized milk becomes separated into whey and curd, and the curd is slowly digested. The first evidence of these enzymatic activities is the appearance of a thin layer of clear whey just at the surface and below the pseudozoogloeal surface growth. As the curd forms, it slowly settles. Cultures three weeks old will have cleared with at most a small quantity of curd at the bottom of the culture tube.

LITMUS MILK.—Lavender-colored litmus milk undergoes the same type of clearing and separation into whey and curd as does plain milk. Little or no free acid is formed, or at least is not formed in sufficient quantity to change the color of the indicator.

CARBON METABOLISM.—In the preliminary tests on the fermentative ability of the soybean pustule organism in which only the common sugars were employed, it was apparent that it could not by this means be separated from *Bacterium phaseoli*. Accordingly, use was made in addition of certain rare sugars which have been successfully employed in distinguishing *Bact. glycineum* and *Bact. sojae* (*11*) and also *Bact. sojae* and *Bact. trifoliorum* (*7*). Bouillon to which was added sufficient of the carbon compounds to make a 1 per cent solution was employed to follow the progressive changes in hydrion concentration as an index to fermentative activity. The sugar was added to cooled, sterile bouillon flasked in convenient quantities and was tubed with aseptic precautions in previously sterilized test tubes. All were made from the same stock bouillon and a considerable number of cultures of each strain were made on the same day. This made possible the employment of several tubes of each strain at each reading at each of the consecutive intervals. The results of these fermentation tests are assembled in Tables I to IV.

TABLE I.—*Fermentation of various carbon compounds by the organisms from soybean pustule (initial P_H 7.0)*

Carbon compound	Age of culture and P_H concentration				
	3 days	5 days	7 days	11 days	13 days
Dextrose	7.0	7 0	7.0	7.4	7.6
Saccharose	7.0	7.0	7.0	7.4	7.6
Lactose	7.0	7.0	7.0	7.2	7.4
Maltose	7.0	7.0	7.0	7.2	7.4
Glycerin	7.0	7.0	7.2	7.6	7.8
Arabinose	7.0	7.0	7.0	7.2	7.4
Xylose	7.0	7.0	7.2	7.4	7.6
Rhamnose	7.0	7.0	7.2	7.6	7.8
Dextrin	7.0	7.0	7.0	7.4	7.6
Salicin	7.0	7.0	7.0	7.4	7.6
Mannitol	7.0	7.0	7.0	7.6	7.8
Inulin	7.0	7.0	7.2	7.6	7.8
Galactose	7.0	7.0	7.2	7.4	7.8

TABLE II.—*Fermentation of the same various carbon compounds by Bact. phaseoli from snap bean (initial P_H 7.0)*

Carbon compound	Age of culture and P_H concentration				
	3 days	5 days	7 days	11 days	13 days
Dextrose	7.0	7.0	7.2	7.2	7.4
Saccharose	7.0	7.0	7.2	7.2	7.4
Lactose	7.0	7.0	7.2	7.6	7.6
Maltose	7.0	7.0	7.0	7.2	7.4
Glycerin	7.0	7.0	7.2	7.4	7.6
Arabinose	7.0	7.0	7.2	7.4	7.6
Xylose	7.0	7.0	7.2	7.4	7.6
Rhamnose	7.0	7.0	7.2	7.4	7.6
Dextrin	7.0	7.0	7.2	7.4	7.6
Salicin	7.0	7.0	7.2	7.4	7.6
Mannitol	7.0	7.0	7.2	7.4	7.6
Inulin	7.0	7.0	7.2	7.4	7.6
Galactose	7.0	7.0	7.2	7.4	7.6

TABLE III.—*Fermentation of various carbon compounds by Bact. phaseoli from Lima bean (initial P_H 7.0)*

Carbon compound	Age of culture and P_H concentration				
	3 days	5 days	7 days	11 days	13 days
Dextrose	7.0	7.0	7.2	7.4	7.6
Saccharose	7.0	7.0	7.2	7.4	7.6
Lactose	7.0	7.0	7.2	7.4	7.6
Maltose	7.0	7.0	7.2	7.4	7.6
Glycerin	7.0	7.0	7.2	7.4	7.6
Arabinose	7.0	7.0	7.0	7.2	7.4
Xylose	7.0	7.0	7.2	7.4	7.6
Rhamnose	7.0	7.0	7.2	7.4	7.8
Dextrin	7.0	7.0	7.2	7.4	7.6
Salicin	7.0	7.0	7.2	7.4	7.6
Mannitol	7.0	7.0	7.2	7.4	7.8
Inulin	7.0	7.0	7.2	7.4	7.8
Galactose	7.0	7.0	7.2	7.2	7.4

TABLE IV.—*Fermentation of various carbon compounds by strain of Bact. phaseoli from Washington, D. C. (initial P_H 7.0)*

Carbon compound	Age of culture and P_H concentration				
	3 days	5 days	7 days	11 days	13 days
Dextrose	7.0	7.0	7.0	7.2	7.4
Saccharose	7.0	7.0	7.0	7.2	7.4
Lactose	7.0	7.0	7.0	7.2	7.4
Maltose	7.0	7.0	7.0	7.2	7.4
Glycerin	7.0	7.0	7.0	7.4	7.4
Arabinose	7.0	7.0	7.0	7.2	7.4
Xylose	7.0	7.0	7.0	7.2	7.4
Rhamnose	7.0	7.0	7.0	7.2	7.4
Dextrin	7.0	7.0	7.0	7.2	7.6
Salicin	7.0	7.0	7.0	7.2	7.6
Mannitol	7.0	7.0	7.0	7.2	7.4
Inulin	7.0	7.0	7.0	7.4	7.6
Galactose	7.0	7.0	7.0	7.2	7.4

It is evident from an analysis of the data presented in these tables that the soybean pustule organism and the several strains of *Bact. phaseoli* are identical so far as concerns their inability to utilize any of these carbons as the source of energy and that they can not therefore be separated on their fermentation relations. The increase in alkalinity, as appears in all cultures with all sugars, develops from the decomposition of the proteins, as shown by growth in plain bouillon.

GAS PRODUCTION.—These tests were conducted by using fermentation tubes filled with portions of the same solutions as were used in the fermentation tests. These tubes were sterilized prior to filling and were incubated for 48 hours after being filled to determine their freedom from contamination. They were then inoculated in sets of four with each of the several strains. No gas was developed in the case of any of the 13 carbon compounds, and growth was sharply limited to the open arm in all media with each of the strains.

NITROGEN METABOLISM.—Unfortunately, no considerable significance has been attached to the nitrogen metabolism of bacteria in relation to the determination of species. This matter has been based largely upon their fermentative ability. It would appear that plant pathogenic bacteria, especially those which attack few or no carbon compounds, as is the case with those under consideration, might be separated upon the basis of differences in nitrogen metabolism were methods of study known. The writer's attempts in this direction have been limited to the employment of a few nitrogenous compounds, added as nutrients to a stock synthetic agar. This agar was prepared according to the following formula: Distilled water, 1,000 cc.; magnesium sulphate, 0.5 gm.; dipotassium hydrogen phosphate, 1.0

gm.; potassium chloride, 0.5 gm.; ferrous sulphate, 0.01 gm.; agar, 20 gm.

DIGESTION OF CASEIN.—Evidence in addition to that obtained in milk cultures of the ability of the organism from soybeans and *Bact. phaseoli* to digest casein was obtained by adding 1 per cent casein to the stock agar in poured plate cultures. After a week's incubation wide halos, in which the casein was entirely dissolved, had formed around the colonies, thus demonstrating the ability of these organisms to form erepsin.

DIGESTION OF ASPARAGIN.—Stock agar plus 1 per cent of asparagin was used in these tests. The indicator consisted of a sufficient quantity of 4 per cent solution of rosolic acid, and sufficient NaOH was added to give the medium a decided orange color and a reaction of about P_H 6.0. In poured plate cultures incubated for about 10 days, the orange color gave way to a beautiful brilliant red. This change begins with a halo around each colony and comes to involve the entire plate. The change in color is due to the liberation of ammonia in the decomposition of asparagin as a result of the activity of the enzym amidase.

DIGESTION OF SERUM.—Blood serum added to stock agar in poured plate cultures was planted with the several strains of bacteria. This medium in cultures 7 to 10 days old serves as a satisfactory means of demonstrating the ability of these organisms to liquefy blood serum.

LIQUEFACTION OF GELATIN.—Growth in stab cultures on gelatin is slow, but within a period of two weeks the gelatin to a depth of about a centimeter will have become liquefied. Liquefaction begins as an infundibuliform area.

REDUCTION OF NITRATES.—Nitrate broth consisting of 1 per cent peptone, 0.3 per cent beef extract, and 0.1 per cent potassium nitrate supports abundant growth. No indication of nitrites was secured when the tests at appropriate intervals were made with sulphanilic-acid solution or with naphthylamine acetate solution.

INDOL PRODUCTION.—No indication of indol was secured by either the Salkowski, Vanilin, or Ehrlich test.

THERMAL DEATH-POINT.—In determining the thermal death-point, tubes of bouillon P_H 6.6 were inoculated from vigorously growing bouillon cultures and subjected for 10 minutes in the usual manner to various trial temperatures. As a result of these tests, and under these conditions, the thermal death-point was found to be 50°C.

RESISTANCE TO DESICCATION

An inoculum of the soybean organism from cultures on potato cylinders 48 hours old was suspended in sterile water and drops of this suspension were transferred to sterile cover glasses kept in sterile Petri dishes. After desiccation at laboratory temperatures certain of these cover glasses were, at definite intervals, inserted into tubes of nutrient broth. Growth appeared after 18 days' desiccation, hence the organism may be regarded as very resistant to drying.

PATHOGENICITY

Only a few pathogenicity trials were made. Pure cultures from young transfers on potato agar were suspended in sterile water, and this bacterial suspension was applied with an atomizer. When inoculations were made late in the afternoon and the plants were covered until the following morning with bell jars, a large number of centers of infection developed. The first evidence of infection was noted within four to six days, appearing as tiny elevations. Within a week later these had developed into typical lesions. From these the original organism was reisolated in trials with several of the strains. Plants in all stages of development from those with the first pair of true leaves to plants with mature foliage are subject to infection with pure cultures. Both garden beans and Lima beans have been inoculated in the same manner, and in some cases with a portion of the same inoculum; but no evidences of infection have been observed on these inoculated plants.

When *Bacterium phaseoli* was employed as an inoculum on Phaseolus and on soybeans, an abundance of typical bean-blight lesions developed upon both the foliage and pods of Lima bean and of garden bean but no evidence of pathogenicity to soybeans was noted.

Miss Hedges has reported (*4*, *5*) the production of infection on soybeans and several varieties of garden beans following inoculation with pure cultures of the soybean pustule organism. The spots on Phaseolus were like those caused by *Bact. phaseoli* with no evidence of pustule formation such as occurs on soybean. She furthermore has found that *Bact. phaseoli* from Phaseolus is only weakly pathogenic to soybean.

The differences in regard to cross inoculation are no doubt due to conditions of inoculation. This opin-

ion is supported by the fact that it has been impossible to separate the soybean organism from bean blight morphologically and culturally. It seems advisable, therefore, to regard the soybean organism as a variety of *Bact. phaseoli.*

RELATION OF PARASITE TO HOST TISSUE

Lesions which were appropriately fixed in alcohol, embedded, sectioned, and stained with alcoholic methylene blue were employed in histological studies. Entrance of the parasite is very manifestly effected through the stomates which occur on both leaf surfaces, as evidenced by the fact that the substomatal chamber and the intercellular spaces of the tissues immediately surrounding the stomata in the case of young lesions are densely filled with bacteria (fig. 4). In the older lesions, after the host cells have collapsed, the bacteria are not confined to the intercellular spaces, but occur within the cell cavities. These phenomena are entirely in accord with those known to occur in the case of nearly all plant bacterial pathogens which invade parenchyma. So far as pustule formation is concerned, however, the writer's observations are not in agreement with the statement made by Miss Hedges (*4*) that the pustules show both hypertrophy and hyperplasia. The preparations in hand show that these elevations arise wholly from hypertrophy and without hyperplasia. Any or all of the tissues, epidermal, palisade parenchyma and mesophyll, may be involved. The physiological phenomena which attend these hypertrophic changes are dependent no doubt upon certain enzymatic activities which involve both the cell walls and the cell contents. The parasite and host relationship and thus the proximate cause of pustule formation is believed to be analagous to that which obtains in the case of citrous canker caused by *Pseudomonas citri* (*15*). In the case of this organism evidence has been advanced that both cytolytic and diastatic enzyms are secreted. Through their activity these modify the osmotic properties of invaded cells and thereby are responsible for their enlargement.

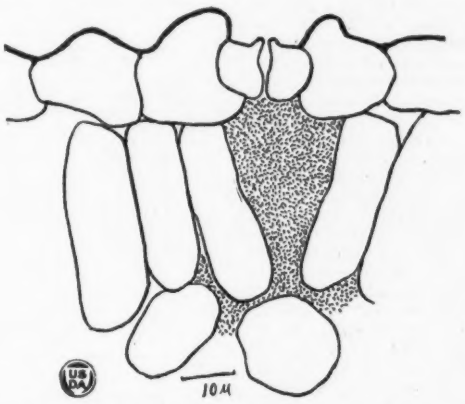


FIG. 4.—Invasion of substomatal cavity by *Bact. phaseoli* var. *sojense*

RÉSUMÉ OF SALIENT CHARACTERS

On the basis of the foregoing studies, *Bact. phaseoli* var. *sojense* is a rod-shaped organism occurring singly, in pairs, or catenulately: The cells measure 1.3 to 2.0×0.6 to .75 μ, are motile by means of a single polar flagellum, possess no well-defined capsule nor endospores, are strictly aerobic and Gram-negative. Colonies on nutrient agar are circular, raised, smooth, shiny, yellow and have an entire or slightly lobed margin. The organism is capable of liquefying gelatin and blood serum, digesting casein and asparagin, is strongly diastatic, very resistant to drying, nonnitrate reducing, and forms neither acid nor gas from the various carbon compounds. Its thermal death-point is approximately 50° C. It has not been possible in culture to distinguish it from *Bact. phaseoli.* According to the 1920 descriptive chart

of the Society of American Bacteriologists its group number is 5322–31135–1333.

SUMMARY

(1) The present investigation concerns a leafspot disease of soybean called bacterial pustule, which is generally prevalent in North Carolina. It is known to occur also in Texas, Louisiana, South Carolina, Virginia, and Kansas.

(2) The disease is distinct from bacterial blight and from the diseases of bacterial origin which have been described in the Orient.

(3) Bacterial pustule appears to be confined to the foliage. Lesions are manifested by the presence of pustular outgrowths on either or both leaf surfaces. They are light green at first, but at maturity collapse and become dry and reddish brown, and the tissues surrounding the lesions become chlorotic.

(4) The disease is caused by an organism to which the name *Bacterium phaseoli* var. *sojense* was first tentatively given by Miss Hedges. This organism is herein fully described and found to be morphologically and culturally indistinguishable from *Bact. phaseoli* E. F. Smith. It forms yellowish colonies on nutrient agar, is flagellate, is unable to utilize any of the carbon compounds tested except starch but can utilize a number of proteins including gelatin, casein, blood serum, and asparagin. According to the descriptive chart of 1920 of the American Society of Bacteriologists, its group number is 5322–31135–1333.

(5) When the organism in watery suspension is applied to uninjured soybean, foliage infection is evident within four to six days. Under the same conditions of inoculation garden beans and Lima beans failed to become infected.

(6) The parasite gains entrance through the stomates and passes thence into the intercellular spaces. The pustules arise by hypertrophic changes of any of the parenchymatous tissues.

LITERATURE CITED

(1) COERPER, F. M.
1919. BACTERIAL BLIGHT OF SOYBEAN. Jour. Agr. Research 18: 179–194, illus.

(2) CROMWELL, R. O.
1917. FUSARIUM-BLIGHT, OR WILT DISEASE, OF THE SOYBEAN. Jour. Agr. Research 8: 421–439, illus.

(3) ———
1919. FUSARIUM BLIGHT OF THE SOYBEAN AND THE RELATION OF VARIOUS FACTORS TO INFECTION. Nebr. Agr. Exp. Sta. Research Bul. 14, 43 p., illus.

(4) HEDGES, F.
1922. BACTERIAL PUSTULE OF SOYBEAN. Science 56: 111–112.

(5) ———
1924. SOYBEAN PUSTULE. COMPARATIVE STUDIES WITH BACTERIUM PHASEOLI SOJENSE HEDGES AND BACTERIUM PHASEOLI E. F. S. (Abstract) Phytopathology 14: 27–28.

(6) ———
1924. A STUDY OF BACTERIAL PUSTULE OF SOYBEAN, AND A COMPARISON OF BACT. PHASEOLI SOJENSE HEDGES WITH BACT. PHASEOLI [EFS]. Jour. Agr. Research. 29: 235–258, illus.

(7) JONES, L. R., and others.
1923. BACTERIAL LEAFSPOT OF CLOVERS. Jour. Agr. Reasearch 25: 471–490, illus.

(8) LEHMAN, S. G.
1923. POD AND STEM BLIGHT OF SOY BEAN. Ann. Mo. Bot. Garden 10: 111–178, illus.

(9) MANNS, T. F.
1915. SOME NEW BACTERIAL DISEASES OF LEGUMES AND THE RELATIONSHIP OF THE ORGANISMS CAUSING THE SAME. Del. Agr. Exp. Sta. Bul. 108, 44 p., illus.

(10) MIURA, M.
1921. DISEASES OF THE MAIN AGRICULTURAL CROPS OF MANCHURIA. Agr. Exp. Sta. South Manchuria Railway Co. Bul. 11, 56 p., illus. [In Japanese]

(11) SHUNK, I. V., and WOLF, F. A.
1921. FURTHER STUDIES ON BACTERIAL BLIGHT OF SOYBEAN. Phytopathology 11: 18–24, illus.

(12) SMITH, E. F.
1904. BACTERIAL LEAF SPOT DISEASES. Science 19: 417–418.

(13) ———
1920. AN INTRODUCTION TO BACTERIAL DISEASES OF PLANTS. 688 p., illus. Philadelphia.

(14) TAKIMOTO, S.
1921. BACTERIAL SPOTTING DISEASE OF SOYBEAN. Jour. Plant Protection 8: 237–241. [In Japanese].

(15) WOLF, F. A.
1916. CITRUS CANKER. Jour. Agr. Research. 6: 69–100, illus.

(16) ———
1920. BACTERIAL BLIGHT OF SOYBEAN. Pytopathology 10: 119–132, illus.

(17) ———, SHUNK, I. V., and FOSTER, A. C.
1921. STUDIES ON THE PHYSIOLOGY OF SOME PLANT PATHOGENIC BACTERIA. N. C. Agr. Exp. Sta. Tech. Bul. 20, 47 p., illus.

THE CHEMICAL EXAMINATION OF VARIOUS PEAT MATERIALS BY MEANS OF FOOD STUFF ANALYSES [1]

By A. P. DACHNOWSKI, *Physiologist, Soil Bacteriology Investigations, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

Several studies have been made in the past few years, and are still being conducted, with the object of accumulating information concerning fundamental differences in peat lands and their relation to agriculture. Prominent among these studies are those dealing with type profiles of peat deposits; that is, with the number of different kinds of peat, their position and arrangement relative to one another, in deposits in different parts of the country. Results thus far secured lead to the conclusion that any adequate description of peat land and the several layers composing it must recognize three fundamental facts: (1) The differences in type of peat and the profile position of the materials; (2) the water level in its relation to the surface zone of oxidation and the lower zone of reducing action; (3) nature of the mineral subsoil and the water supply affecting the quantity and character of salts, such as lime, iron, sulphur, etc.

CHEMICAL ANALYSES OF VARIOUS KINDS OF PEAT

The following study was undertaken to obtain data on the differences in the principal groups of organic compounds of several types of peat layers and to establish, if possible, standards for grading types of peat as to stage of decomposition and relative agricultural value. In determining the relative usefulness of different kinds of peat, for example, to microorganisms and higher plants, it is necessary to find adequate means of indicating the amount of different organic substances which the various layers of peat actually contain. It is obviously important to know what variation may be expected in the layers of peat in different regions of the country in varying stages of decomposition, and at different depths. Likewise it is evident that the composition of any layer of peat, whether stated in terms of calories, of soluble organic material, or of insoluble residue, contributes toward a basis for judgment and for securing standards of value for commercial peat products.

The simplest means for compiling chemical composition records are analyses as made for crops and feeding stuffs, in accordance with official methods (1)[2]. The results obtained in regard to the amount of crude protein, crude fiber, nitrogen-free extract, and fat present in different peats can be readily compared with those derived from analyses of plants and feeding stuffs.

The results reported here [3] indicate that data of this kind are probably fully as important as those secured by other means for determining changes and losses of organic constituents in peat materials varying with composition, stage of decay, and depth below the surface. In many particulars, however, the grouping under these terms of organic substances in different kinds of peat is unsatisfactory. The method does not give a sufficiently forceful illustration of the available organic compounds in plant remains now stored as layers of peat. It would, therefore, be preferable to differentiate between the several nitrogenous substances, carbohydrates and fats, and substitute a more accurate, though necessarily more complicated classification.

Tables I and II represent the chemical composition of samples from typical layers of peat obtained over a great portion of this country. A number of these types of peat have been illustrated in a recent paper (*9*).

[1] Received for publication Apr. 9, 1924—issued January, 1925.
[2] Reference is made by number (italic) to "Literature cited," p. 81-83.
[3] The analytical work on this series of peat samples was done by G. L. Bidwell of the Cattle Food and Grain Investigation Laboratory, Bureau of Chemistry, U. S. Department of Agriculture.

TABLE I.—*Foodstuff analyses of various peat materials*

Type of peat	Locality	Condition	Depth below surface	Inorganic matter		Organic matter			
				Water	Ash	Crude protein	Crude fiber	Nitrogen-free extract	Fat
I. SEDIMENTARY			*Ft.*						
Colloidal	Fremont, Ind	Gelatinous	4	2.58	65.48	9.63	12.21	9.81	0.29
Macerated	Lake Okeechobee, Fla.	Finely divided	1	9.81	32.67	15.75	14.19	27.43	.15
Do	Middle River, Calif.	Coarsely divided	2	9.40	22.39	13.25	15.25	38.48	1.23
II. FIBROUS									
Sedge (*Carex* sp.) peat	Chelsea, Mich	Coarsely fibrous	4	7.71	4.97	17.81	30.16	36.51	2.84
Do	Corona, Minn	Finely fibrous	6	8.99	18.67	13.63	23.82	33.79	1.10
Do	Deerfield, Wis	Fibrous, partly carbonized.	3	6.14	10.88	18.69	20.89	42.46	.94
Sedge (*Cladium* sp.)	Okeelanta, Fla	Coarsely fibrous	2	11.24	7.20	19.63	33.20	28.48	.25
Sedge (*Scirpus* sp.)	Middle River, Calif.	Fibrous, partly decomposed.	2	9.19	16.06	14.56	16.80	42.52	.87
Reed (*Phragmites* sp.)	Plymouth, Ohio	Fibrous	3	8.74	3.42	13.13	21.82	52.18	.71
Do	do	"Muck," well decomposed.	0	10.97	16.65	17.69	9.05	45.20	.44
Do	Wood County, Wis	Fibrous, slightly decomposed.	2½	8.29	5.31	11.69	17.78	53.15	3.78
Brown moss (*Hypnaceae* sp.)	Phillips, Wis	Fibrous, partly decomposed.	5	9.84	19.77	9.81	11.71	46.63	2.24
Do	Algoma, Miss	Fibrous	2	10.76	7.54	13.94	23.19	43.82	.75
Bog Moss (*Sphagnum* sp.)	Fairbanks, Alaska	Coarsely fibrous	1	9.66	3.80	4.03	41.86	38.99	1.66
Do	Culver, Minn	Fibrous	1	9.82	2.54	2.75	40.69	42.73	1.47
Do	Calais, Me	Fibrous, partly decomposed.	2	9.43	1.07	4.38	30.21	52.94	1.97
III. WOODY									
Heath shrub and sedge	Charlevoix, Mich	Partly decomposed	2	8.17	2.98	9.94	20.81	54.94	3.16
Forest peat (coniferous)	Margie, Minn	do	3½	10.81	6.38	10.13	24.67	46.08	1.93
Do	Charlevoix, Mich	do	1	13.97	18.89	10.44	17.55	37.66	1.49
Forest peat (mixed)	Winamac, Ind	Largely decomposed.	2½	10.82	22.84	10.69	15.70	39.51	.44

TABLE II.—*Relation of carbonaceous to nitrogenous material in different types of peat examined by means of foodstuff analyses—calculated on a moisture and ash free basis*

Type of peat	Locality	Depth below surface	Organic matter				Ratio of C to N*
			Crude protein	Fiber	Nitrogen-free extract	Fat	
I. SEDIMENTARY		*Feet*					
Colloidal	Freemont, Ind	4	30.15	38.23	30.71	0.91	2.2
Macerated	Lake Okeechobee, Fla.	1	27.38	24.67	47.69	.26	2.6
Do	Middle River, Calif.	2	19.43	22.36	56.41	1.80	4.0
II. FIBROUS							
Sedge (*Carex* sp.)	Chelsea, Mich	4	20.40	34.54	41.81	3.25	3.7
Do	Corona, Minn	6	18.84	32.93	46.71	1.52	4.2
Do	Deerfield, Wis	3	22.52	25.17	51.18	1.13	3.1
Sedge (*Cladium* sp.)	Okeelanta, Fla	2	24.07	40.71	34.91	.31	3.0
Sedge (*Scirpus* sp.)	Middle River, Calif.	2	19.48	22.47	56.89	1.16	4.0
Reed (*Phragmites* sp.)	Plymouth, Ohio	3	14.95	24.84	59.40	.81	5.6
Reed "Muck" (*Phragmites* sp.)	do	0	24.44	12.50	62.45	.61	3.0
Reed (*Phragmites* sp.)	Wood County, Wis	2½	13.53	20.58	61.51	4.38	6.0
Brown moss (*Hypnaceae*)	Phillips, Wis	5	13.94	16.64	66.24	3.18	5.9
Do	Algoma, Minn	2	17.06	28.38	53.64	.92	4.8
Bog moss (*Sphagnum* sp.)	Fairbanks, Alaska	1	4.66	48.37	45.05	1.92	20.0
Do	Culver, Minn	1	3.14	46.43	48.75	1.68	30.3
Do	Calais, Me	2	4.89	33.75	59.15	2.21	19.0
III. WOODY							
Heath shrub and sedge	Charlevoix, Mich	2	11.19	23.41	61.84	3.56	7.6
Forest peat (coniferous)	Margie, Minn	3½	12.23	29.79	55.65	2.33	6.9
Do	Charlevoix, Mich	1	15.55	26.14	56.09	2.22	5.2
Forest peat (mixed)	Winamac, Ind	2½	16.11	23.67	59.56	.66	5.1

* The terms C and N in this column are used as a numerical expression of the relation of crude fiber and nitrogen-free extract to crude protein.

At this time only a few of the principal groups of plant products can be considered, and but a few examples of the chief kinds of peat material have been examined. This new matter, however, offers a basis for reviewing the results of some of the more recent peat investigations made by various workers. Although the aims of sectional workers are divergent, their contributions are necessarily interdependent. Tentatively these and the results of the present series of analyses are summarized and discussed in relation to (1) nitrogenous substances in various types of peat; (2) nonnitrogenous substances, including crude fiber and nitrogen-free extract; (3) ether-alcohol extract; and (4) mineral matter.

The search for new facts concerning the physical, chemical, and bacteriological qualities of the various layers of peat should take into account all the basic facts and conclusions which are of importance to practical workers in peat land utilization.

NITROGENOUS SUBSTANCES IN DIFFERENT PEAT MATERIALS

The nitrogenous substances in peat materials are of interest agriculturally and industrially in several ways. One to five per cent of organic nitrogen may be found in peat, but little of this is available as plant food. Jodidi (*21*), Stutzer (*50*), and others have shown that nitrates are not present in freshly dug peat materials. The quantity of nitrogen as ammonia is very small, ranging from a few thousandths to a few hundredths of 1 per cent of nitrogen. The water solubility of peat nitrogen varies within wide limits. More nitrogen can be extracted from a sample of peat in a finely divided state than from a coarse fibrous or woody type of material. Peat digested at higher temperature and pressure in an autoclave gives a larger amount of water-soluble nitrogen. The amounts and concentration of acid or alkaline reagents, the duration of digestion, as well as other methods of manipulation in the laboratory have an influence upon the percentage of nitrogenous bodies that can be extracted from peat materials. The relative value of peat materials containing high amounts of these compounds depends on the means by which the nitrogenous substances in peat layers can be extracted by distillation and obtained as ammonia, or otherwise can be converted into an available source of plant food. It has long been known that a composting procedure of some kind must be adopted to make peat nitrogen easily accessible either for crops grown upon peat land, or for plants grown on mineral soils to which peat is applied for the production of humus.

The nitrogenous substances in peat materials are very complex, because in addition to the nitrogenous constituents of plants, bacteria, and molds, the layers of peat also contain the remains of animals of various kinds. On account of their great variety and complexity little more than a beginning has been made in determining the differences in the composition of nitrogenous bodies in organic materials. The extreme complexity and variety of the organic compounds in soils has been demonstrated mainly through the work of Schreiner and his collaborators (*46*). Only recently, however, investigations have been undertaken upon the nature of these compounds in the different kinds of peat and upon the variations in the availability of their nitrogen.

In peat the crude protein may consist of a large proportion of inert substances of animal origin. A very considerable part of this fraction in sedimentary types of peat is frequently due to the admixture of chitinous compounds derived from certain seeds, fungi, insects, the egg cases and skeletal portions of various crustaceous, and other forms of plankton life. Birge and Juday (*3, p. 31*) report that crude fiber derived from plankton crustacea yielded 5.9 to 6.2 per cent of nitrogen. This material is relatively unhydrolyzable and has a low decomposition value.

In air-dried peat materials, the crude protein constitutes from 2.75 per cent to 19.63 per cent of the organic matter (Table I). The results show clearly that the range of variation is largest in the fibrous group of peat materials. Sphagnum peats yield the smallest percentage, varying from a little more than 2 to about 4.5 per cent. In comparison with these the sedge and reed peats yielded over 11 to 19.63 per cent. On the other hand, woody and sedimentary types of peat average only about 11 per cent.

Table II shows the variations in crude protein when stated on a moisture and ash-free basis. The maximum of 30.15 per cent is found in the gelatinous peat from Fremont, Ind., and the minimum percentage in the sphagnum peats from Culver, Minn., Fairbanks, Alaska, and Calais, Me. Peats of the sedge, reed, and woody types contain from 12 to 24 per cent of crude protein. Taken as a whole, the results show distinct dif-

ferences in the percentage of crude protein among the three chief groups of peat materials. There is a considerable increase in the amount of crude protein, from 14.95 to 24.44 per cent, in the reed peat from Ohio in its raw condition, and after it has been transformed into "muck" by cultivation.

In the present state of our knowledge concerning the particular type of nitrogenous compounds in different types of peat the term crude protein is inadequate to cope with the accumulated facts. For reasons given in the following section not much significance can be attached to the amounts of crude protein obtained, at least in so far as the agricultural use of this material is concerned.

The proteins and amides are subdivided on the basis of the nature of their products of hydrolysis and upon the solubilities of the individual compounds. For a rough division the nitrogen insoluble in lead-acetate solution is considered protein nitrogen. The nitrogen that is evolved as ammonia upon treatment with acid, subsequently distilled by potash but not by calcium carbonate, is considered as amide nitrogen. However, it is not yet well understood to what extent these terms may be applied to the fractions obtained from the different kinds of peat.

A review dealing with the study of proteins and amides in soils and peat is available in the literature given below; hence it is unnecessary to go into any detail here. As early as 1844 Mulder (*34*), continuing the investigations made by Einhof, Sprengel, and others, pointed out that the organic nitrogenous material in soils is largely of protein origin. Later, Detmer (*10*) observed that a considerable portion of this nitrogen could be liberated by adding nitrous acid. Jodidi (*21*) determined the relative quantities of mono- and diamino-acid nitrogen together with the nitrogen obtained as ammonia from a fibrous sedge peat. Similarly Suzuki (*51*) reported that he isolated such compounds in a material derived from peat. Robinson (*38*) found that treatment of peat with acid, and lengthening the time of hydrolysis increased the amount of amino nitrogen, until it reached a constant maximum. Robinson concluded that in comparison with ordinary proteins the amino group of peat nitrogen did not exist free but in some resistant form of combination which differed radically from the acid amides. Gortner (*16*), working with three different but undetermined kinds of peat, obtained a maximum of 7.5 per cent of total nitrogen soluble in 1 per cent hydrochloric acid, and an average of 3.78 per cent; in five samples of unchanged vegetable materials (oat straw, alfalfa hay, oak leaves, sweet-fern leaves and grasses from a peat bog) he found a maximum of 34.58 per cent and an average of 20.10 per cent. According to Gortner, these findings would indicate that in the humification of vegetable materials there is a decrease in total nitrogen soluble in very dilute acids. Later, Morrow and Gortner (*33*) studied the amounts of the various forms of nitrogen isolated from samples of sphagnum peat and from material to be identified, it seems, as sedge and sedimentary types of peat. It is quite possible that the material designated by the authors as "sphagnum peat and subsoil" would be found to consist of a layer of sphagnum on sedge peat, and that the "calcareous black peat" is of pond-formed origin, while the analyzed "muck" is sedge peat under cultivation.

Miller and Robinson (*39*) divided the acid soluble nitrogen in peat materials into ammonia nitrogen, acid amide nitrogen, and amino nitrogen, calling attention to the fact that the largest two fractions are the acid amides and the monoamino acids. They further point out that, contrary to a statement frequently found in the literature, there is no regular increase in nitrogen content with depth or age of the peat material, the fluctuations being determined rather by differences in the botanical composition of the various layers of peat. These results are in accordance with those of Zailer and Wilk (*53*), Bersch (*2*), Minssen (*32*), and others, outlined in Bulletin 802 (*8*).

In regard to the decomposition of peat materials, the results of both American and Eurasian peat investigations are of much interest. They indicate that during the accumulation of plant remains as peat, the protein bodies are profoundly altered. Continued submersion of layers of peat in water renders difficult the conversion of their nitrogen into ammonia. A high water level precludes both oxidation of the compounds involved and favorable bacterial activity. When submerged, the transition of the plant remains to peat materials is usually a rapid one; this also accounts for the greater stability of peat nitrogen. Many of these substances remain unavailable even after the water-logged layers of peat have been drained and aerated. Some of the nitrogen may be split off by heating, steaming, or treating the

peat material with acids or alkalis. But at present this method is not an economical one. By composting peat with manure, sewage, or other plant and animal waste products, however, a relatively large portion of the peat nitrogen may be made soluble, and may be mineralized by the action of bacteria and fungi. Thus far the bacteriology of this process has received but scant attention.

NONNITROGENOUS SUBSTANCES IN VARIOUS KINDS OF PEAT

Carbohydrates constitute the major portion of the organic material in plants as well as in peat, and they form one of the most important sources of energy for microorganisms. The carbohydrates include the lignose or woody portion of plant products, the cellulose with related forms, the pentoses corresponding to sugars, the pentosans usually associated with starches and celluloses, and many nonnitrogenous acids. Although certain groups of plants produce a characteristic diversity of carbohydrates, yet all these substances are closely related chemically, being composed of the same elements.

Due to the comparatively large amount of carbonaceous compounds in layers of peat, the fermentation and carbonization of this type of raw organic materials is advocated. Carbonized peat acts as an absorbent for uncrystallized residues from beet and cane-sugar refining. Charred material of this kind has been used in the United States as an ingredient in the preparation of commercial feed with cottonseed meal and molasses, thus permitting the feeding of large quantities of these materials to livestock and poultry. It is claimed that properly prepared, carbonized peat contains valuable nutritive ingredients and acts as a corrective and preventive in livestock diseases. The investigations of Kellner (*23*), Goy (*17*), Stutzer (*50*), Godden (*14*), and others have shown that as a matter of fact peat does not have any particular nutritive value when used as a basis for stock feed, whether in raw condition, treated with acid, or carbonized.

The possibility of using dried and charred peat as a diluent with mineral fertilizers or with tankage, and the use of peat innoculated with bacteria in compounding of commercial fertilizers is also a matter of considerable interest. Much depends upon adequate general information, and upon devising new and more effecient methods for determining the grade and value of the several kinds of peat material used in such products.

The course of decomposition of peat layers through bacterial action is greatly influenced by the relative content and quality of nonnitrogenous and nitrogenous substances. In regard to the carbon-nitrogen ratio Zailer and Wilk (*53*) and Bersch (*2*) have found that the relation of these two elements does not change with the depth of a peat deposit, but varies with the botanical composition of the peat-forming plant remains. The conclusion to be drawn from the results reported in Tables II and III is in accordance with these authors. As in other cases, discussed below, peat deposits with an irregular and complex pedomorphic profile show no regularity between carbon-nitrogen value and depth or age of the peat material. However, when a deposit is examined which has the same botanical composition throughout, the investigations show a tendency toward the attainment of a maximum value of carbon and nitrogen, and a minimum for oxygen and hydrogen.

Little is known regarding the complex of factors which would enable one to designate peat materials as superior, good, fair, or poor in quality. In this country it has not been determined experimentally whether peat materials with a wide carbon-nitrogen ratio are more or less valuable than those with a narrow ratio of carbonaceous and nitrogenous material. Information presented by Löhnis (*26*) makes it very probable that peat materials with comparatively large quantities of carbonaceous compounds and relatively low amounts of nitrogen are not as advantageous as those in which the relation of nitrogen to carbon is a narrower one.

The data in Table II indicate that under similar conditions peat layers derived from sedges, reeds, hypnum mosses, and woody plant remains in which the average relation of C:N is equal to 6 or 10:1 are of a better grade and would make better "muck" during soil-forming processes than the layers of sphagnum peat in which the relation of nonnitrogenous to nitrogenous matter is as 20:1. These results also show that the sedimentary, finely divided pond-formed layers of peat possess a very narrow range of about 2C:1N. The largest variation is in the fibrous group of peat materials.

These facts support the information conveyed in Bulletin 802 (*8*) relating to the economic rank or value and the

relative agricultural possibilities of the several types of peat in this country. The presence of a narrow carbon-nitrogen ratio in sedimentary kinds of peat, as in the case of intensively cultivated fibrous and woody "muck" soils, may be considered to show not only advanced decomposition but also a deficiency in fresh carbonaceous materials. To restore a more balanced ratio, an application of green manure might be advantageous. On the other hand, organic materials of a wide ratio, such as represented by the sphagnum type of peat, may not favor decomposition processes or an increase in nitrogen except upon the addition of peat materials of much narrower carbon-nitrogen ratio or upon the application of liquid stable manure, tankage, sewage sludge, or mineral fertilizers supplying more soluble nitrogen.

Since carbonaceous substances constitute by far the greater part of the organic matter in peat, the relative importance of some of the different forms of nonnitrogenous compounds deserves consideration. In the present method of classification the carbohydrates are grouped as crude fiber and nitrogen-free extract.

Crude fiber is the term applied to the more resistant lignose and cellulose substances of plant products. It is determined by digesting a sample successively with weak acid and alkali (1.25 per cent) and by washing out the soluble material. What remains undissolved, free from ash, is termed crude fiber.[4] The analytical data obtained on various types of air-dried peat are shown in Table I. A very considerable portion of the fibrous light types of peat derived from mosses, sedges, and reeds, constitutes crude fiber. The largest percentage is 41.86, found in the sphagnum peat from Alaska. Partially disintegrated phases of the fibrous group of peat contain relatively less fiber. The well-decomposed and cultivated "muck" derived from reed peat yields the minimum of 9.05 per cent. The decrease noticeable in the amount of crude fiber varies with the degree of decomposition rather than with age or depth of the peat layer below the surface. To a great extent, the plant remains in shrub and forest peat also are fiber; homogeneous structureless peat material of sedimentary origin likewise responds to this test. Finely divided, gelatinous peat and the coarsely macerated water-laid organic matter furnish between 12 to 15 per cent of crude fiber. The differences between the quantity of crude fiber are of similar magnitude when expressed in terms of ash and moisture-free materials (Table II). The amount of crude fiber ranges from a minimum of 12.5 per cent in the reed "muck" from Ohio, to a maximum of 48.37 per cent in the Alaska sphagnum peat. In general, the percentage is comparatively lower the greater the degree of decomposition.

Chemical analyses show that pentosan forms a considerable portion of the fiber component; it is usually associated with cellulose. Peat materials yield measurable amounts of furfural when distilled with hydrochloric acid, the furfural being collected as the phloroglucide, and the result calculated to pentosan. This constituent belongs to a complex less stable towards decay, diminishing in percentage progressively as the peat material becomes chemically altered. Von Feilitzen and Tollens (*12*) have shown that pentosans and methyl pentosans are quite generally present in layers of peat and that sphagnum mosses or the upper and less decomposed horizons of sphagnum peat contained as much as 14.7 per cent and 12.75 per cent of pentosans, respectively. As a rule these high percentages were accompanied by relatively high percentages of nitrogen. Leavitt (*25*) obtained pentosans in appreciable amounts from a peat material of the Florida Everglades. Since the amount of pentosan present in most plant products is roughly in proportion to the amount of fiber, von Feilitzen and Tollens (*12*) suggested the possibility of employing the pentosan determination as a means for indicating quantitatively the degree of decomposition of peat materials. In the light of Rose and Lisse's work (*40*) and the investigation extended by Gorbenko (*15*), it may now be accepted that the assumption is justified by proof. According to Street (*49*), it is possible to adopt the phloroglucin method in use for the determination of pentosans in cattle feed, as a means of detecting the presence of peat in commercial fertilizers.

The outstanding components of crude fiber are lignose and cellulose substances. Both classes of material have been found in peat by various investigators.

The lignose group of organic materials consists of a base of true cellulose

[4] The filtering of the fiber proved to be very difficult on account of the gelatinous nature of the treated peat sample.

which in the course of time has been altered by the addition either of encrusting substances or of chemically combined noncellulose constituents. Hence the lignose materials in plant remains are not definite compounds like cellulose, but vary in composition owing to the thickening of the cellulose surface by adsorption of different substances from the sap in trees, shrubs, and other woody plants. The composition of lignin has not been definitely determined; it is more resistant to decay and difficult to hydrolyze. Lignin is obtained by treating wood with strong hydrochloric acid in accordance with the method of Willstätter and Zechmeister (*52*). The insoluble portion contains a methoxyl-yielding complex when treated with concentrated hydrochloric acid in the manner originally described by Zeisel (*54*). Methoxyl (CH_3O) has been determined in the wood of a large number of trees by Ritter and Fleck (*37*), while Shorey and Lathrop (*48*) found it present in the organic matter of widely different mineral soils. But the fact that its occurrence bears a relation to lignocellulose tissue indicates that the quantity of methoxyl in the organic matter of mineral soils and in that of different layers of peat varies with respect to the kind of vegetation that is the source of the lignose compounds, and with their persistence during the process of decay. As the decomposition of woody material progresses, and the less stable cellulose disappears, an increase will become apparent in the methoxyl content. In the Velen peat deposit, which has been studied by Fischer and his coworkers (*13*), the quantity of methoxyl in sphagnum moss peat is less than that in the woody layers of peat; the sedimentary organic material from greater depths shows a corresponding increase in the methoxyl content of the portion insoluble in acid. The results recorded by Fischer are important also in that they show by the action of solvents a general agreement between the botanical composition of peat materials and their content in bitumen and in alkali-soluble material. The German investigators point out that the methoxyl content does not vary necessarily with increasing depth but that it fluctuates, exhibiting, however, a tendency toward the attainment of a maximum value in woody plant remains. The results confirm the earlier work of Rose and Lisse (*40*).

Cellulose is very common in the plants from which peat is derived. There are a great many varieties of cellulose, and hence the term must be taken as denoting a group (*6*). Evidence of the existence of cellulose in peat materials has been obtained by von Feilitzen and Tollens (*12*). Comparative analysis showed that the cellulose content decreases during decomposition. The samples contained from 6.64 to 15.37 per cent of cellulose, those taken from layers below the surface of a peat deposit yielding the smaller percentage. Sphagnum moss contained from 20.8 to 21.42 per cent of cellulose.

It has long been known that cellulose could be broken down into reducing sugar by treatment with acids. Keppeler (*24*) using a strong solution of sulphuric acid (72 per cent), developed a method of hydrolysis for determining the degree of decomposition of peat layers. Tests made with this method showed that sphagnum peat of recent origin is greatly hydrolyzed and hence has a very low degree of decomposition, scarcely exceeding 25 per cent. Samples of sphagnum peat of older origin were less strongly hydrolyzed, the degree of decomposition varying between 70.9 and 78.8 per cent. Tests of the profile of a peat deposit indicated that the degrees of decomposition increased with the depth below the surface of the deposit. A comparison of the Keppeler method with that of von Feilitzen (*12*) leads Gorbenko (*15*) to the conclusion that on peat materials of Russian deposits the Keppeler method is inaccurate and too complicated, probably owing to the differences in the profiles of the peat deposits and to the difficulty of removing the product of hydrolysis.

More recently, Schneider and Schellenberg (*44*) reported briefly on the action of different solvents for cellulose in peat. These authors observed that samples of various kinds of peat treated with carbon bisulphide and sodium hydroxide (Xanthogenic acid), or with the ammoniacal cupric oxide (Schweitzer's reagent) show a moderate solvent effect. The yields of insoluble residues are not materially less than those obtained by treatment with alkali alone. The cellulose of peat materials appears to be more resistant in alkalies, but the proportion of organic material soluble in alkali, referred to as humic acids, is much larger in the deeper and older layers of peat (*45*). The action of reagents such as ammonia and sodium hydroxide is noteworthy in that it points more clearly to the differences connected with the botanical composition of the respective layers of peat. It would seem, therefore, that the

differing facility of hydrolysis in the peat materials is to be related to the different origin and character of the carbonaceous material, rather than to depth or any subsequent alterations of the peat material in the deposit.

Results obtained by H. M. Cooper of the Pittsburgh, Pa., Experiment Station of the United States Bureau of Mines in the analysis of samples collected in cooperation with the Michigan Land Economic Survey in Charlevoix County, are presented in Table III, IV, and V. The peat materials were taken from three different type-profile deposits. Each of these had the same botanical composition throughout, so that any marked changes in the material due to variation in the composition of the peat-forming plants were eliminated. The carbonization and extraction tests on the three principal groups of peat were carried to 550° C. maximum temperature. The samples were carbonized in air-dried condition and crushed to pass a 60-mesh sieve. The gas obtained was practically free from light oils. The work so far carried on with type materials respectively from sedimentary, fibrous, and woody peat indicates clearly a definite variation in the character and the amounts of the thermal products from carbonization, corresponding with the differences in the origin and the botanic composition of the peat material, obtained at whatever depth.

Aside from the great interest which a high content of fiber and of the cellulose group of substances in peat may have for the industries, their dominant value agriculturally is due to the fact that carbonaceous compounds are the food supply and the source of energy for microorganisms in the soil.

TABLE III.—*Proximate and elementary analysis on different peat materials, air-dried, from Charlevoix, Mich.*

Components	Sedimentary aquatic peat	Fibrous sedge peat	Woody forest peat
Moisture	6.1	7.5	12.0
Ash	21.6	4.6	19.2
Volatile matter	57.9	61.1	47.8
Fixed carbon	14.4	26.6	21.0
Carbon	40.0	51.7	39.6
Nitrogen	3.4	2.2	1.9
Oxygen	28.0	34.7	33.5
Hydrogen	5.6	6.0	4.9
Sulphur	1.4	.8	.9
Calories	4,044	4,972	3,583

TABLE IV.—*Carbonization and extraction tests on various types of peat material from Charlevoix, Mich.*

Type of peat	Dry tar and water-soluble salts	Water	Ammonia	Gas, cubic feet per ton	Residue	Alcohol soluble	Ether soluble
	Per cent	*Per cent*	*Per cent*		*Per cent*	*Per cent*	*Per cent*
Sedimentary	12.5	24.3	.....	3,560	45.7	0.42	0.18
Fibrous sedge	9.4	25.9	.....	4,595	41.3	1.38	.71
Woody forest	4.7	23.6	0.075	4,760	51.6	.84	.38

TABLE V.—*Analysis of gas obtained from carbonization tests on different types of peat from Charlevoix, Mich.*

Type of peat	Unsaturated hydrocarbons	Oxygen	Hydrogen	Nitrogen	Carbon monoxide	Carbon dioxide	Methane	Ethane
Sedimentary	4.5	0.5	14.3	4.1	8.2	39.9	19.5	9.0
Fibrous sedge	2.2	.6	11.0	4.3	13.3	39.7	25.6	3.0
Woody forest	1.8	1.3	14.4	11.6	8.3	44.6	14.3	3.6

Crop production on peat deposits is dependent on processes leading to the decomposition of carbonaceous materials. Hence, the biological phenomena which transfer cellulose to soluble compounds are obviously of fundamental importance. But investigations upon quality and quantity of available carbonaceous compounds in different types of peat are still problematical. It is certain that bacteria as well as fungi are present in definite horizons of peat deposits, and that both act differently in the several kinds of peat. Microorganisms which make use directly of cellulose or live on its decomposition products may depress or favor the accumulation of nitrogen to a marked degree. An accurate knowledge of the biochemical value of the various layers of peat from different depths and stages of decomposition, and the soluble products formed from them under water-logged conditions and during soil-forming processes, is of great practical value. The rapidity with which the cellulose component becomes decomposed may indicate the differences in the fertility of peat land. The investigations undertaken by Kellerman and his collaborators (*22*, *29*) on the destruction of cellulose by bacteria and filamentous fungi have done much to stimulate this phase of the peat problem. The work of Löhnis and his students (*26*, *28*, *41*) to determine the nutritive value of peat humus for Azotobacter, the studies of Hutchinson and Clayton (*20*), Christensen (*5*) and others, have demonstrated the widespread occurrence of cellulose-destroying bacteria and that they usually act symbiotically, utilizing probably intermediate products. It would be desirable in this connection to ascertain more accurately the degree of utilization of specific peat materials by different bacteria and fungi. Based on observations made in the field, certain fungi are unique in the way their mycelium incloses completely portions of fibrous or woody layers of peat at depths from 2 to 4 feet below the surface, and form clusters of fruiting bodies in the space formerly occupied by the peat material. No reference covering these observations has been found in the literature.

In view of the fact that the present classification of carbonaceous substances in peat materials includes intermediate products of decomposition in the nitrogen-free extract, it is important to determine the amount and the distribution of this fraction in different layers of peat.

The nitrogen-free extract, as given in Tables I and II, composes all carbonaceous residues free from fiber, crude protein, fatty substances and ash. The quantity of this fraction is determined by difference. The nitrogen-free extract embraces a great variety of soluble organic substances. In the analyses of crops and feeding stuffs this fraction represents the starches, sugars, pentoses, nonnitrogenous acids and similar compounds, including glucosides, pectins, and tannins. It is unfortunate that for this group of substances at present peat investigators must rely upon figures obtained "by difference." The extent of the analytical error as well as the actual composition of this fraction in the different types of peat remain unknown.

The analyses in Table I show that the nitrogen-free extract in air-dry peat materials varies from 9.81 per cent, in the Fremont, Ind., gelatinous peat, to 54.94 per cent in the heath-shrub peat with fibrous sedge admixture obtained from Charlevoix, Mich. Most samples contain 30 to 40 per cent. The two samples from the Florida Everglades show a relatively low percentage, approximately 27.8 per cent. The small proportion of nitrogen-free extract in the Florida fibrous sedge peat, and in certain other peat samples, is doubtless to be explained by the loss of dispersed and colloid organic material in the progressive leaching characteristic of humid regions. Calculated on a moisture and ash-free basis this fraction of organic matter ranges from a minimum of about 30.71 per cent in the colloidal peat from Fremont, Ind., and of 34.91 per cent in the leached fibrous peat from Florida, to a maximum of 66.24 per cent in the hypnum peat from Phillips, Wis. In the majority of peat samples, however, it amounts to 40 and 50 per cent. Again the quantity of the nitrogen-free extract exhibits a tendency to vary not with depth below the surface, but with the composition of the plant remains and their relative stage in chemical alteration. As shown in Tables I and II, the more decomposed reed, sedge, and sphagnum types of peat, irrespective of their age or position in the profile of a deposit, yield more than 50 per cent of substances classed as nitrogen-free extract, though some of the component materials are undoubtedly lost by leaching. Coarsely fibrous sedge and sphagnum peat contain much less of this fraction. Woody and sedimentary peat material, high in fiber, also supply only moderate amounts. The results suggest strongly that the state of decomposition does not run parallel to the depth at which a peat material is found below the surface, but that an approximate correlation exists be-

tween the botanical composition of the layer of peat and its relative stage of decay. If this conclusion is further substantiated, the quantitative estimation of the nitrogen-free extract in different layers of peat materials should be of special significance and value. The quantity of this fraction of organic material must influence the character of cultural operations and the rate of growth of crops in the peat-soil horizons through which roots can penetrate. It is quite reasonable to assume that the nonnitrogenous components in the separate layers or horizons in peat deposits may be responsible for the differences in the water-holding capacity of peat materials and in the absorption power for certain mineral salts and fertilizers. Changes of this kind may likewise contribute to layers with varying salinity, and to the stratification of acid and alkaline reaction in certain peat-land profiles. The horizons and pockets of well-preserved shells of mollusks (*9, Pl. 1, 4*) in submersed fibrous and sedimentary types of peat, reacting acid to litmus, appear to indicate that the organic acids present are only slightly soluble in water and act upon calcareous materials with great difficulty.

Undoubtedly a more detailed examination of peat layers in regard to their nitrogen-free extract would shed much light upon their microbiological character and upon the biochemical changes going on during the humification of peat materials. Under natural conditions of high-water level, that is, when reduction prevails, the solubility of the substances grouped into this fraction is apparent in the color imparted to the streams which emerge from marshes, bogs, and swamp forests. The content of water-soluble organic matter varies probably with the pedomorphic structure of the peat deposit and with the relative capacity for chemical alterations of the several layers of peat. The color of the solution obtained from freshly dug peat samples varies with different stages of decomposition, ranging from a light straw-yellow to a deep brown. Freezing and heating or steaming different peat materials are accompanied by marked changes in the content of dispersoid and colloid organic material. Studies are reported by Ostwald (*36*) and by Melin and Odén (*30*), which indicate the possibility of using a colorimetric method for investigations of this kind.

Frequently organic substances in peat, forming suspensions and colloidal solutions with water, have a strongly acid reaction and absorb oxygen from the air. Little is known as to their direct effects on plants, although by former investigations (*7*) it was shown that such aqueous peat extracts may contain organic substances of harmful character. A tendency was observed to inhibit or retard and dwarf the growth of higher plants as well as of aerobic microorganisms. The question is still an open one, however, whether some of the organic substances can be absorbed by plants and can be used as sources of carbon.

The dilute alkali extract of peat materials and of the organic matter in mineral soils has been the object of numerous studies on organic decomposition. It is not the purpose of the present paper to review or to discuss the voluminous literature dealing with this phase of the problem. The results of investigations on humus, humic acids, on theories of adsorption and related phenomena have been comprehensively reviewed by Löhnis (*26*), Ehrenberg (*11*), Hoering (*19*), and more recently by Odén (*35*). But with reference to the several kinds of peat and the marked stratigraphic differences in peat deposits, the situation remains problematical. Neither the relation of microorganisms to the formation of muck and humus is understood, nor is the chemical composition of the organic compounds in the nitrogen-free extract of specific types of muck and peat a well-known subject. It should be highly instructive to determine whether the increase in nitrogen-free extract is due largely to the decomposition of cellulose and lignin complexes or to that of other plant products. Attempts should be made therefore leading to a more detailed analysis and to the isolation of compounds from the nitrogen-free extract. Indeed the change toward the formation of such substances is so marked that a comparative cellulose and lignin determination of various peat materials undergoing decay might well serve to throw further light upon the problem of humus.

THE ETHER EXTRACT IN DIFFERENT PEAT MATERIALS

Ether-alcohol extract of peat materials contains dissolved waxes, variable mixtures of gummy resins, balsams, terpenes, and similar substances together with other complex constituents. They are storage, waste, and residual products of very variable chemical structure, and most of them were originally secreted by cells and glands of different kinds of plants. A study of solvents for the separation of these substances from peat materials with par-

ticular reference to the differences in the action of benzol, toluol, xylol, and phenol, as well as of ether and alcohol, is just beginning to be made (*42*).

Many of the extracted substances, such as, "fichtelite," "ozokerite," and others (*19*) do not belong to the true fats and oils. Fatty substances when breaking down yield a considerable series of fatty acids without the acid H-ion. They give off more heat on burning, because they contain a small proportion of oxygen but relatively more carbon and hydrogen than the carbohydrates. Waxes and resinous substances are especially abundant in the foliage of woody heaths and evergreen trees. Hence, peat layers derived from these plants carry more of the materials extracted by ether and other solvents than the peats derived from sedges, reeds, or mosses. Treatment of peat with phenol yields more extract than with any other solvent, and the yields are considerably increased by the use of higher temperatures (*43*.) The products, moreover, have considerably higher melting points than the other extraction products. The extracts resemble more or less the bitumin from lignites. Schneider and Schellenberg (*42*) found that the action of solvents depends also on the relative age of the peat material, the deeper and older layers in a given peat profile with the higher proportion of resistant material giving the higher yields. The evidence presented by them is, however, insufficient, and the error in the conclusion has been demonstrated by the work of Zailer and Wilk, and others cited in Table I of Bulletin 802 (*8*).

There are marked differences in the percentages of ether-alcohol extract obtained from the several air-dried peat materials of this country. In general, the extract figures shown in Table I vary from 0.15 per cent in the Lake Okeechobee, Fla., sedimentary peat to 3.78 per cent in the reed peat from Wood County, Wis., which underlies a conifer forest vegetation and shrub heaths as ground cover. The same range of variation in the percentages of this fraction is, furthermore, seen in Table II where the results are stated on an ash- and moisture-free basis. The finely divided sedimentary peat from Lake Okeechobee, Fla., contains a minimum of 0.26 per cent and the Wood County, Wis., reed peat yields the maximum of 4.38 per cent. Together with the figures in Table IV these results again indicate that the botanical composition of the plant remains forming peat layers is a considerably more important factor than the depth below the surface. In the sedge peats from Chelsea, Mich., as well as in the sphagnum peats from Fairbanks, Alaska, and from Calais, Me., the presence of relatively large amounts of ether-alcohol extract is probably due to an admixture in the peat samples of leached plant residue from heath shrubs. There is a tendency towards the attainment of a maximum value for the ether-alcohol fraction, which appears to be more or less closely correlated with the degree of decomposition of the peat material. The data in Tables I and II show that increasing accumulation of the more resistant organic material may cause a steady increase in the waxy, resinous residue. The analyses for the partly decomposed hypnum peat from Phillips, Wis., for the woody peats from Margie, Minn., and from Charlevoix, Mich., and for the sedge and sphagnum peats which have undergone a partial decay, agree in showing this tendency. Thus the percentages of ether-alcohol extract in the several peat materials depend not only upon the type of the plant remains, but also on the stage of alteration and upon the predominant admixture of distinctly heath components.

INORGANIC MATTER IN DIFFERENT PEAT LAYERS

Mineral matter is present in peat materials either incorporated in organic compounds, in cell walls, as crystals and incrustations, or held by the absorptive power of the finely divided organic matter. Foreign material constitutes not infrequently the larger portion of the ash content in certain classes of peat deposits. It is brought in during the accumulation of peat layers by wind and water as dust, silt, or clay, and through the growth of shell mollusks, diatoms, sponges, and other organisms. The inorganic contamination may be disregarded for the present, since it is not a part of plant ash or that of peat, although reported as such in peat analysis. In most investigations concerning the agricultural value of peat land, the ash and its "standard fertilizer" ingredients are the only factor considered in grading peat areas. However, the quantity and character of the mineral matter vary from point to point in the same deposit, and even in the same layer. Owing to the constant evaporation of water from the surface of cultivated peat areas, many of the salts accumulate at or just below the surface peat soil. The amount and constituents vary much, because the presence of mineral matter, such as

clusters of calcium sulphate crystals, nodular concretions of iron, marl, and surface concentrations of lime or alkali, are determined largely by the chemical nature of circulating ground waters, by overflow, or by the excess of soluble salts carried in the mineral subsoils. In order to guard against a wrong interpretation of field data, it is necessary to remember, therefore, that the chemical nature of the groundwater or of the geological formation of a region does not in itself determine the fertilizer needs of peat land in that region.

The ash of the peat materials, indicated in Table I, consists of the total mineral matter left when the organic matter is completely burned. The differences in ash content range from 1.07 per cent in the sphagnum peat from Calais, Me. to 65.48 per cent in the Fremont, Ind., gelatinous material. The figures for the three samples of bog moss peat, for the Charlevoix, Mich., heath shrub and sedge peat, and for the Wisconsin reed peat indicate that a considerable loss of mineral matter has taken place by leaching. The sedimentary types of peat, respectively from Fremont, Ind., Middle River, Calif., and Lake Okeechobee, Fla., show a relatively high ash content; this is to be correlated, undoubtedly, with the general siltation of the water basins from which these samples have been obtained. The ash content of the cultivated reed "muck" from Plymouth, Ohio, and of the drained and aerated woody forest peats from Charlevoix, Mich., and Winnamac, Ind., exhibits the influence of soil-forming processes; the increase during decomposition is due partly to the adsorptive power of the finely divided organic components, but mainly to the transformation of organic matter into carbon dioxide.

Interesting information on the ash constituents of plants and peat materials is reviewed by Hoering (*19*). The recent work of Hibbard (*18*) indicates that the ash of "tule" (*Scirpus lacustris* var. *occidentalis*) is similar to kainit as a source of potash, but that the leached plant remains in the California tule peat lands have lost most of the mineral material worth recovering. Miller (*31*) gives data upon the inorganic composition of sawgrass (*Cladium effusium*) from the Florida Everglades. But the evidence submitted by him regarding layers of peat supposedly resulting from the accumulation of the remains of sawgrass is palpably insufficient. The thorough and accurate studies made by Zailer and Wilk (*53*), Bersch (*2*), Birk (*4*), and Minssen (*32*) show the changes of the inorganic material in different kinds of plants forming peat and in the corresponding layers of peat derived from them. These authors give a large number of analyses; they also point out that the ash content does not increase with the depth of the deposit, but varies with the nature of the peat profile, and with the contamination by extraneous sand, silt, or lime. In general, the work conducted by these and other investigators shows that silica is undoubtedly one of the most stable mineral constituents when present in a peat layer, while potash and to a less extent, phosphorus leach readily from different types of peat. On the other hand, it has not been emphasized sufficiently that iron and sulphur compounds are among those which not infrequently affect extensive areas of otherwise desirable peat land and may cause root rots. The deposition of dissolved iron in plant remains and its gradual replacement of the organic matter has been shown in a former publication (*8, Pl. 1*). The injurious effect of the accumulation of soluble iron within the tissues of growing plants reported by Sherwin (*47*) further emphasizes the necessity of modifying the accepted fertilizer standard of ash analyses.

Continued submersion in water makes various mineral salts comparatively unstable in peat. The darker color of peat samples taken from greater depths below the surface, the production of sulphuretted hydrogen and of methane, the reduction of sulphates and possibly of phosphates (vivianite) together with those other changes which are characterized by the withdrawal of oxygen not only from the water but also from the carbonaceous and nitrogenous substances in the organic matter, indicate the nature of the reduction action (*7*). The absorptive power for certain mineral substances appears to be localized in finely divided peat. The relative "saturation" of its colloidal constituents probably influences to a considerable extent the character and the varying salinity of the peat soil solution, the kind and concentration of various bases, and the rate at which microorganisms and filamentous fungi decompose peat materials. The low mineral salt content of certain types of fibrous peat soils in all probability accounts for the lack of nutrition in the grasses of such peatland pastures. Further work is greatly needed to determine the movement, absorption, and the losses and exchange of soluble mineral material in layers of

woody, fibrous, and sedimentary peat at different depths, as well as during the transformation of drained surface horizons to muck and humus.

CONCLUSIONS

Results obtained with 20 different kinds of peat indicate the feasibility of the application of foodstuff analyses for the determination of qualitative differences in sedimentary, fibrous, and woody peat materials. These data point to contrasts between peat materials in regard to their nitrogenous substances, lignin, cellulose, and other carbohydrates, waxes, resins, and similar plant products. Their value is necessarily limited, since it is only possible to utilize analyses of the same character as those used for crops and feeding stuffs. However, they illustrate the fact that a close connection exists between the botanical and the chemical composition of the main groups of peat. By the application of this method it becomes possible to correlate variations in the chief groups of organic compounds with structural differences in the profile of peat deposits, to follow the progress of decomposition in drained surface peat soils, and to determine the degree of chemical alteration taking place in the several layers of peat below the water level. The analyses show the wide differences in agricultural value of the several kinds of peat, on the basis of the ratio of nonnitrogenous to nitrogenous materials, and the limited usefulness of various kinds of raw peat as a source of food for livestock or for soil microorganisms.

LITERATURE CITED

(1) Association of Official Agricultural Chemists.
1920. Official and tentative methods of analysis. As compiled by the committee on revision of methods. Revised to Nov. 1, 1919. 417 p., illus. Washington, D. C.

(2) Bersch, W.
1907. Die moore oesterreichs. Eine botanisch-chemische studie. Ztschr. Moorkultur u. Torfverwertung 5: 175–196, 343–374, 429–473.

(3) Birge, E. A., and Juday, C.
1922. The inland lakes of wisconsin. I: the plankton, its quantity and chemical composition. Wis. Geol. and Nat. Hist. Surv. Bul. 64, 222 p., illus.

(4) Birk, C.
1914. Das tote moor am steinhuder meer. Arb. Lab. Tech. Moorverwertung K. Tech. Hochschule Hannover 1: 1–102, illus.

(5) Christensen, H. R.
1914. Studier over jordbundsbekaffenhedens indflydelse paa bakterielivet og stofomsaetningen i jordbunden. Tidsskr. Planteavl 21: 321–552, illus.

(6) Cross, C. F., and Dorée, C.
1922. Researches on cellulose iv. (1910–1921). 253 p., illus. London, New York, etc.

(7) Dachnowski, A. P.
1912. Peat deposits of ohio. Geol. Surv. Ohio, Ser. 4, Bul. 16, 424 p., illus.

(8) ———
1919. Quality and value of important types of peat material. U. S. Dept. Agr. Bul. 802, 40 p.

(9) ———
1924. The stratigraphic study of peat deposits. Soil Sci. 17: 107–134, illus.

(10) Detmer, W.
1871. Die natürlichen humuskörper des bodens und ihre landwirtschaftliche bedeutung. Landw. Vers. Stat. 14: 248–300.

(11) Ehrenberg, P.
1918. Die bodenkolloide. 717 p., illus. Dresden and Leipzig.

(12) Feilitzen, H. von, and Tollens, B.
1898. Über den gehalt des torfes an pentosan und anderen kohlen hydraten. Jour. Landw. 46: 17–22.

(13) Fischer, F., Schrader, H., and Friedrich, A.
1922. Über den methoxylgehalt vermodernder pflanzenstoffe. Gesam. Abhandl. Kenntnis der Kohle (1920) 5: 530–540.

(14) Godden, W.
1920. Digestibility of peat moss after treatment with acid. Jour. Agr. Sci. 10: 457–459.

(15) Gorbenko, V.
1922. Zur frage der bestimmung von torfzerlegungs grade. Mitt. Wiss. Exp. Torf-Inst. Moskau 2: 104–121. [In Russian. German résumé, p. 121.]

(16) GORTNER, R. A.
1916. THE ORGANIC MATTER OF THE SOIL: I. SOME DATA ON HUMUS, HUMUS CARBON, AND HUMUS NITROGEN. Soil Sci. 2: 395–441, illus.

(17) GOY, S.
1914. ZUR FRAGE DER VERDAULICHKEIT DES TORFES. Landw. Jahrb. 46: 403–408.

(18) HIBBARD, P. L.
1917. POTASH FROM TULE AND THE FERTILIZER VALUE OF CERTAIN MARSH PLANTS. Calif. Agr. Exp. Sta. Bul. 288, p. 187–192.

(19) HOERING, P.
1915. MOORNUTZUNG UND TORFVERWERTUNG. 638 p. Berlin.

(20) HUTCHINSON, H. B., and CLAYTON, J.
1919. ON THE DECOMPOSITION OF CELLULOSE BY AN AEROBIC ORGANISM (SPIROCHAETA CYTOPHAGA, N. SP.). Jour. Agr. Sci. 9: 143–173, illus.

(21) JODIDI, S. L.
1909. ORGANIC NITROGENOUS COMPOUNDS IN PEAT SOILS. Mich. Agr. Exp. Sta. Tech. Bul. 4, 28 p., illus.

(22) KELLERMAN, K. F., et al.
1913. IDENTIFICATION AND CLASSIFICATION OF CELLULOSE-DISSOLVING BACTERIA. Centbl. Bakt. (II) 39: 502–522, illus.

(23) KELLNER, O., ZAHN, V. O., and GILLERN, H.
1901. FÜTTERUNGSVERSUCHE MIT MELASSE UND TORFMEHL. Landw. Vers. Stat. 55: 379–388.

(24) KEPPELER, G.
1920. BESTIMMUNG DES VERTORFUNGSGRADES VON MOOR UND TORFPROBEN. Jour. Landw. 68: 43–70, illus.

(25) LEAVITT, S.
1912. STUDIES ON SOIL HUMUS. Jour. Indus. and Engin. Chem. 4: 601–604.

(26) LÖHNIS, F.
1910. HANDBUCH DER LANDWIRTSCHAFTLICHEN BAKTERIOLOGIE. 907 p. Berlin.

(27) ——— and GREEN, H. H.
1914. ÜBER DIE ENTSTEHUNG UND DIE ZERSETZUNG VON HUMUS, SOWIE ÜBER DESSEN FINWIRKUNG AUF DIE STICKSTOFFASSIMILATION. Centbl. Bakt. (II) 40: 52–60.

(28) ——— and LOCHHEAD, G.
1923. EXPERIMENTS ON THE DECOMPOSITION OF CELLULOSE BY AEROBIC BACTERIA. Centbl. Bakt. (II) 58: 430–434.

(29) MCBETH, I. G., and SCALES, F. M.
1913. THE DESTRUCTION OF CELLULOSE BY BACTERIA AND FILAMENTOUS FUNGI. U. S. Dept. Agr. Bur. Plant Indus. Bul. 266, 52 p., illus.

(30) MELIN, E., and ODÉN, S.
1917. KOLORIMETRISCHE UNTERSUCHUNGEN ÜBER HUMUS UND HUMIFIZIERUNG. Sveriges Geol. Undersökning, Arsbok 10, No. 4, 46 p., illus. Reviewed in Internat. Mitt. Bodenk. 9: 391–418, illus. 1920.

(31) MILLER, C. F.
1918. INORGANIC COMPOSITION OF A PEAT AND OF THE PLANT FROM WHICH IT WAS FORMED. Jour. Agr. Research 13: 605–609.

(32) MINSSEN, H.
1913. BEITRÄGE ZUR KENNTNIS TYPISCHER TORFARTEN. (VORLAUFIGE MITTEILUNG.) Landw. Jahrb. 44: 269–330.

(33) MORROW, C. A., and GORTNER, R. A.
1917. THE ORGANIC MATTER OF THE SOIL: V. A STUDY OF THE NITROGEN DISTRIBUTION IN DIFFERENT SOIL TYPES. Soil Sci. 3: 297–331.

(34) MULDER.
1844. UEBER DIE BESTANDTHEILE DER ACKERERDE. Jour. Prakt. Chem. 32: 321–344.

(35) ODÉN, S.
1919. DIE HUMINSÄUREN. Kolloid-Chem. Beihefte 11: 75–260.

(36) OSTWALD, W.
1921–22. BEITRÄGE ZUR DISPERSOIDCHEMIE DES TORFES I–III. Kolloid Ztschr. 29: 316–329, 1921; 30: 119–133, 1922; 31: 197–200, 1922; illus.

(37) RITTER, C. J., and FLECK, L. C.
1923. CHEMISTRY OF WOOD. VI. Indus. and Engin. Chem. 15: 1055–1056.

(38) ROBINSON, C. S.
1911. ORGANIC NITROGENOUS COMPOUNDS IN PEAT SOILS. II. Mich. Agr. Exp. Sta. Tech. Bul. 7, 22 p., illus.

(39) ——— and MILLER, E. J.
1917. ORGANIC NITROGENOUS COMPOUNDS IN PEAT SOILS: III. Mich. Agr. Exp. Sta. Tech. Bul. 35, 29 p., illus.

(40) ROSE, R. E., and LISSE, M. W.
1917. THE CHEMISTRY OF WOOD DECAY. PAPER I, INTRODUCTORY. Jour. Indus. and Engin. Chem. 9: 284–287.

(41) SCHMIDT, E. W.
1920. TORF ALS ENERGIEQUELLE FÜR STICKSTOFFASSIMILIERENDE BAKTERIEN. Centbl. Bakt. (II) 52: 281–289.

(42) SCHNEIDER, W., and SCHELLENBERG, A.
1922. ÜBER DEN BITUMENGEHALT DES TORFES. Gesam. Abhandl. Kenntnis der Kohle (1920) 5: 1–33.

(43) ——— ———
1922. ÜBER DIE EXTRAKTION DES TORFES MIT PHENOL. Gesam. Abhandl. Kenntnis der Kohle (1920) 5: 34–36.

(44) ——— ———
1922. ÜBER DAS VERHALTEN DES TORFES GEGEN VERSCHIEDENE FÜR CELLULOSE CHARAKERISTISCHE LÖSUNGSMITTEL. Gesam. Abhandl. Kenntnis der Kohle (1920) 5: 37–43.

(45) ——— ———
1922. ÜBER DIE EINWIRKUNG VON WÄSSRIGEN ALKALIEN AUF TORF. Gesam. Abhandl. Kenntnis der Kohle (1920) 5: 377–406.

(46) SCHREINER, O.
1913. THE ORGANIC CONSTITUENTS OF SOILS. U. S. Dept. Agr. Bur. Soils Circ. 74, 18 p.

(47) SHERWIN, M. E.
1923. SOIL TREATMENTS TO OVERCOME THE INJURIOUS EFFECTS OF TOXIC MATERIALS IN EASTERN NORTH CAROLINA SWAMP LAND. Jour. Elisha Mitchell Sci. Soc. 39: 43–48.

(48) SHOREY, E. C., and LATHROP, E. C.
1911. METHOXYL IN SOIL ORGANIC MATTER. Jour. Amer. Chem. Soc. 33: 75–78.

(49) STREET, J. P.
1907. THE DETECTION OF PEAT IN COMMERCIAL FERTILIZERS. U. S. Dept. Agr. Bur. Chem. Bul. 105: 83–85.

(50) STUTZER, A.
1915. VERSUCHE UM DIE AUS SPHAGNUMTORF BESTEHENDE TORFSTREU ALS FUTTERMITTEL VERWERTBAR ZU MACHEN. Landw. Vers. Stat. 87: 215–227.

(51) SUZUKI, S.
1907. STUDIES ON HUMUS FORMATION, III. Bul. Col. Agr. Tokyo Imp. Univ. 7: 513–529.

(52) WILLSTÄTTER, R., and ZECHMEISTER, L.
1913. ZUR KENNTNIS DER HYDROLYSE VON CELLULOSE: I. Ber. Deut. Chem. Gesell. 46: 2401–2412, illus.

(53) ZAILER, V., and WILK, L.
1911. DER EINFLUSS DES VERTORFUNGSPROZESSES AUF DIE ZUSAMMENSETZUNG VON CAREXTORF. Ztschr. Moorkultur u. Torfverwertung 9: 153–168, illus.

(54) ZEISEL, S.
1886. ÜBER EIN VERFAHREN ZUM QUANTITATIVEN NACHWEISE VON METHOXYL. Monatsh. Chem. (1885) 6: 989–996.

BOTRYTIS ROT OF THE GLOBE ARTICHOKE [1]

By George K. K. Link, *Pathologist*, Glen B. Ramsey, *Associate Pathologist*, and Alice A. Bailey, *Junior Pathologist, Office of Cotton, Truck, and Forage Crop Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

Gray mold rot causes heavy losses in practically all truck-crop shipments made during the cooler months of the year, especially from California. Cross-inoculation experiments have indicated that the strains of *Botrytis* sp. affecting the Globe artichoke (*Cynara scolymus* L.) are pathogenic to those vegetables subject to decay from the Botrytis usually called *B. cinerea* Pers.

In their survey of plant diseases as they occur on the market, Link and Gardner [2] reported gray mold rot (*Botrytis* spp.) as the only disease affecting the buds of the Globe artichoke in transit and on the market.

The disease has been observed in the United States wherever artichokes have been examined. Practically all the artichokes marketed in this country are grown in Contra Costa and San Mateo Counties, Calif., mostly on the rich loams and clay loams of the narrow strip along the seashore from Montara to Pescadero; some are grown also in the canyons extending from the seashore into the mountains. The buds are shipped in refrigerator, freight, and express cars to eastern markets, 50 per cent of the total shipments going in 1922 to New York.

The economic importance of the Botrytis rot of the artichoke is suggested by abstracts which the plant disease survey made of inspection certificates issued by the food products inspection service of the Bureau of Agricultural Economics. Although the inspection service does not examine all cars arriving terminal in markets, the abstracts of inspections made show the artichoke to be affected by only one rot, which is practically the only cause of loss in transit. Ninety-six per cent of the car-lot inspections made during 1918 to 1923 report losses of 2 to 100 per cent due to Botrytis, the remainder being due to freezing. About 10 per cent of the inspections show losses of 50 per cent and over; 20 per cent show loses of 20 per cent and over; 70 per cent show losses of less than 20 per cent. These losses are considerable since the market price of a car (470 to 480 boxes) is $1,800 to $2,500, depending upon market conditions, with a freight and icing charge of about $375 from California to Chicago. The presence of even slight decay necessitates expensive resorting and considerable loss to wholesalers and retailers. Severely affected buds are a total loss, although slightly affected ones can be sold at a reduced price.

Inquiries from receivers, carriers, and food products inspectors of the Bureau of Agricultural Economics in 1919 to 1922 have shown a prevalent supposition that the serious losses on artichoke shipments were mainly attributable to freezing injury and chilling. There were consequent mutual recriminations among growers, shippers, carriers, and receivers, each believing some other responsible for the occurrence of such injury. None had realized that the great losses in transit might be connected with the presence in the field of a certain fungus.

Reduction of such wastage and loss in transit, with fixing of the responsibility for these, calls for definite knowledge concerning the source and mode of contamination, the time, place, and manner of infection, and the development and spread of the disease. As there were no experimental data adequate for formulating control measures and placing responsibility for losses in vegetables from this source, the artichoke was chosen for investigation of the disease, on account of certain advantages which it affords for type

[1] Received for publication Feb. 28, 1924—issued January, 1925. Paper from the Cooperative Laboratory for the Investigation of Market and Transit Diseases of Vegetables and Fruits, the U. S. Department of Agriculture and the University of Chicago cooperating.

[2] Link, G. K. K., and Gardner, M. W. Market pathology and market diseases of vegetables. Phytopathology 9: 497-520. 1919.

Link, G. K. K., Ramsey, G. B., and Bailey, A. A. Botrytis rot of the globe artichoke (cynara scolymus l.). (Abstract.) Phytopathology 13: 58. 1923.

study, even though this product is less important than the majority of crops affected by the fungus.

The work discussed in the present paper leads to the conclusion that, without previous or attendant freezing injury, a species of Botrytis, to be placed in the *cinerea* group, can produce the condition responsible for the deterioration and consequent losses in transit.

DESCRIPTION OF THE DISEASE

The lesions which indicate the disease are so small and inconspicuous that they are often overlooked in harvesting and packing, the more so since they vary with external conditions.

In the field the lesions are usually but 1 to 2 mm. in diameter on actively growing buds of vigorous plants, and are generally restricted to the tips of the scales. They are sunken and brown to black. Sometimes buds severely affected by Botrytis are found on aging plants, or on vigorous plants after the close of the active period of bud production (October to May). These usually shrivel and wither, and are covered with sporulating mycelium.

Extensive lesions of the moist type can be seen on vigorous plants in the field. They usually start in the wounds made when the buds are cut, whence they progress down the flower stalks and spread to the lateral branches and even to the main stems of the plants.

The lesions may occur anywhere on the bud. Spore infection seems most frequent on the cut stem and at the tips of the scales which are generally somewhat split by growth tensions. Contact with the mycelium leads to infection of any part; under moist conditions the spider weblike growth of the mycelium starting from one original center of infection frequently involves the entire bud, the rot progressing from scale tip to scale tip and down into the scale so that finally the entire bud is destroyed (pl. 1, A, B). Infection at the base of the scales or in the stem may destroy the entire stem and receptacle so that the bud readily falls apart (pl. 1, B). Under dry conditions, an affected bud then becomes a shriveled mummy.

Under moist conditions the lesions on the buds have definite slightly water-soaked borders, semimoist to wet, odorless, reddish-brown to brown, and generally with abundant growth of sporulating mycelium on their older portions. Under drier conditions the advancing edge is not water-soaked, the affected tissues are dry and firm, brown to black, and show no aerial development.

THE CAUSAL ORGANISM

ITS ISOLATION AND IDENTITY

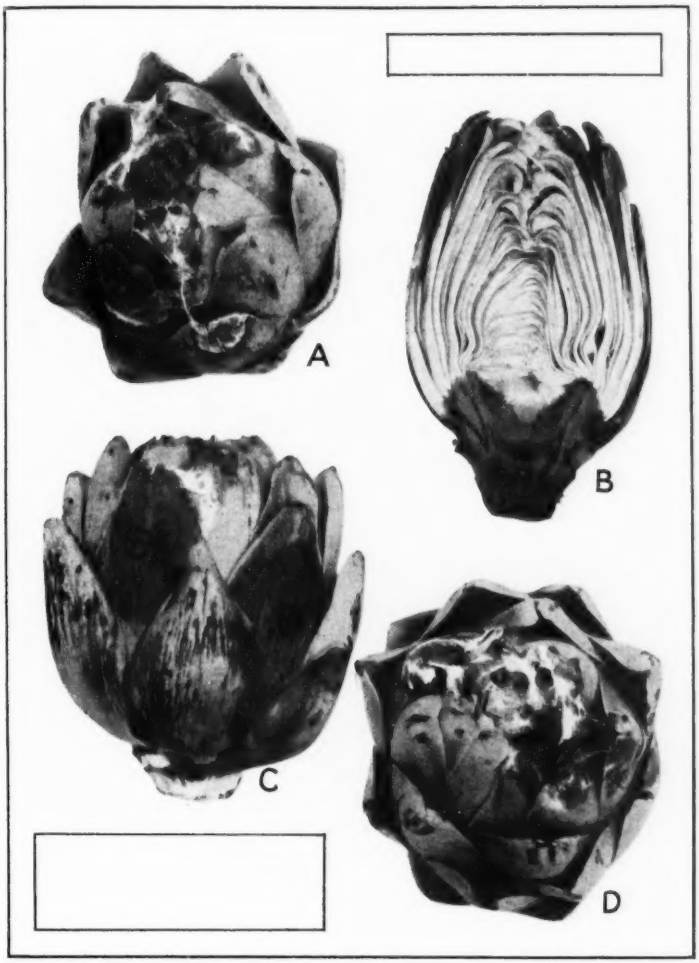
Isolations of *Botrytis* sp. made from the flower buds of artichokes collected on the Chicago market, and from the stalks, stems and buds collected in the fields near Half Moon Bay, Calif., furnished the cultures used in the present study. A lot shipped from the fields was of particular interest because the entire plants including flower buds were frozen solid during transit. Viable cultures apparently identical with strains collected on the market were isolated from this material. One strain isolated from the interior of a frozen stem showing a brown surface lesion has maintained characteristics seeming to indicate a difference from all other cultures. On first isolation plates this culture, No. 255, grew more like a Fusarium than a Botrytis; but after one week's growth sclerotia began to develop and conidia were observed around the edges of the plate. The cultures of this strain always produce a honey-yellow[3] color in the substratum and fine ivory-yellow mycelium on potato-dextrose agar plates, as contrasted with the smoke-gray to light grayish-olive mycelium and grayish-olive substratum of the other strains. The gray strains also make greater vegetative growth and sporulate much more freely and abundantly than the yellowish strain.

Morphologically, there do not seem to be great differences among the cultures under observation. The mycelium is pale smoke-colored at first,

[3] RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C. 1912.

EXPLANATORY LEGEND FOR PLATE 1

A. Artichoke held in a humid atmosphere, showing lesions and spider weblike growth of Botrytis mycelium over the surface.
B.—Longitudinal section through bud showing stem and receptacle infection.
C.—Artichoke showing points of inoculation and lesions resulting therefrom. Mycelium was inoculated into wounds whose position is indicated by circles. Dark discolored areas show limits of fungus advance. The blotched discoloration on lower two scales is due to bruising.
D.—Lesions and mycelium on split and wounded tips of scales resulting from inoculation with a spore suspension.


A
B
C
D

becom
age.
loose
slightl
latera
phore
preser
either
short,
phore

Wit
ture N
slightl
there
the co
the se
studie
10.5μ
length

The
measu
within
the fu
Pers.
sion r
Botry
refer
under
type,
of thi
strain
identi
cinere
tion o

In t
and t
was f
just i
was a
ing fr
at —
which
media
under
in ot
very
differ
expec
vigor
All o
mm.
days.
0° th
radiu
No.
grew
temp
rapid
daily
made
at sti
that t

[4] BR
search

becoming gray to grayish-brown with age. Conidia are produced in rather loose clusters on the rounded to slightly inflated ends of the tips and lateral branches of the upright sporophores. In old cultures sclerotia are present, which on germination give rise either to vegetative mycelium or to short, rather compact tufts of conidiophores with grayish-brown conidia.

With the possible exception of culture No. 255, whose spores are usually slightly smaller than those of the others, there is very little variation in size of the conidia of the various cultures. In the seven cultures used in most of the studies, the spores ranged from 8.2 to 10.5μ in width and from 11.3 to 14.4μ in length, averaging 9.4 by 13.1μ.

The growth characteristics and spore measurements of these fungi are well within the range and description of the fungus usually designated *B. cinerea* Pers. In view of the existing confusion regarding species within the genus Botrytis, it has been thought best to refer to the organism or organisms under consideration as the *B. cinerea* type, since, as stated in the beginning of this article, practically all Botrytis strains occurring on vegetables are identical and can be grouped as the *B. cinerea* type (with the possible exception of culture No. 255).

TEMPERATURE RELATIONS

In temperature experiments on plates and tubes of potato-dextrose agar, it was found that one culture, No. 1610, just isolated from an artichoke bud, was able to make radial growth ranging from 1 to 7 mm. within seven days at −2° C. None of the other strains which had been cultured on artificial media for some time were able to grow under the above conditions, although in other respects the strains appear very similar. This suggests that a difference in pathogenicity may be expected in cultures, due to loss of vigor in growing on artificial media. All other strains grew from 0.5 to 2 mm. in radius at −1.5° to −1° in seven days. During the seven-day period at 0° these strains grew 7 to 14 mm. in radius while the newly isolated strain No. 1610 under parallel conditions grew but slightly faster. At higher temperatures growth becomes more rapid until at 20° to 22° an average daily radial growth of 5 to 7 mm. is made. From experiments conducted at still higher temperatures it appears that the optimum for growth on potato-dextrose agar lies between 22° and 25°. These observations agree well with the results of Brooks and Cooley [4] for *B. cinerea*.

The reactions to temperatures above 31° are approximately alike for the various strains in plate cultures. Usually very little growth is made. An increase of 1 to 2 mm. observed in some cases was mostly a very fine aerial mycelium arising from the inoculum. A few sporophores bearing conidia were also noted developing from the inoculum of one strain. But their vitality is so reduced at the above temperature that the organisms are not likely to produce appreciable decay.

SPORE GERMINATION

The spores of all strains germinate readily in hanging drops of sterile water and on the surface of nutrient agar plates. The majority germinate within 24 hours at room temperature and above, while a smaller percentage form germ tubes at temperatures around 0° C. The extreme range is wide, as successful germination has been obtained at temperatures as low as −2° and as high as 32° C.

An experiment in which the temperatures ranged between −2° C. and −1.5° with a mean of −1.75° showed good germination in the three strains tested. In a similar experiment in which spore dilutions of four different strains were poured upon plates of potato dextrose agar and placed where the temperature varied between −1° and −3°, good germination was obtained within 48 hours. From 50 to 75 per cent of these spores germinated, and their germ tubes ranged from 20 to 90μ in length. It appears from these and other tests that mature conidia falling upon a suitable nutrient medium can germinate and make slow growth at temperatures below 0°. In sterile water at the above temperatures, however, germination seldom occurs, or is at most rather feeble compared with growth on a nutrient medium.

Spore dilutions on potato-dextrose agar plates held at 31° to 32° C. give 50 to 90 per cent germination within 24 hours. Growth of the germ tubes continues 4 or 5 days, then usually stops when they reach 100 to 200μ in length. This would seem to indicate a temperature of 32° as the maximum for growth from conidia in the strains under observation.

[4] BROOKS, C., AND COOLEY, J. S. TEMPERATURE RELATIONS OF APPLE ROT FUNGI. Jour. Agr. Research 8: 139-163, illus. 1917.

PROOF OF PATHOGENICITY

The pathogenicity of the organism was established by its constant association with and isolation from lesions and by numerous inoculation tests made with artichoke and other vegetables.

Vigorous fresh-appearing buds, free from blemishes, were used. They were prepared for the test by making a fresh cut of the stem; by thorough washing; by dipping into 1:1,000 mercuric chloride solution for about a minute followed by thorough rinsing in sterile water. Longer immersion in corrosive sublimate solution was found to cause discoloration of the tender scales. Preliminary experiments and the controls proved the method adequate to remove or kill any spores on the buds.

To duplicate the method of inoculation which seems to occur naturally, both spores and mixtures of spores and mycelium were used as inocula. The spores were suspended in water and the suspension either poured or sprayed on the buds. These methods, especially the latter, approached as nearly as possible to the manner of inoculation in the field. Mycelium inoculation was made by inserting agar inoculum, or infected scales which generally had some mycelium on their surfaces, between the scales of sound buds. This duplicated the mode of inoculation involved when the disease spreads in transit. After inoculation the buds were kept in moist chambers or covered battery jars.

Inoculation experiments with infected scales demonstrated that the fungus can produce decay throughout its temperature range. As the buds freeze at −2° C., no tests were made below this temperature. None of the cultures which had been grown on agar for some time produced appreciable decay at −2°, whereas strain No. 1610, recently isolated from artichokes, produced decay. Four scales of a bud inoculated with this strain became infected to the extent of 6 sq. cm. after a week. The presence of the fungus in the advancing edge of the lesions was proved by microscopic examination and plate cultures. At −1° all strains produced decay. The percentage of successful inoculations increases steadily up to the optimum temperature of 22° to 25° C. Failures were infrequent at this temperature but increased rapidly above 24°; occurred in about 85 per cent of the attempts at 26°; and lesions were obtained but rarely between 26° and 32°.

Although infection was more consistently and rapidly obtained through wounds when mycelium was used as inoculum, the experimental evidence shows that wounds are not a necessity. The inoculum was applied at the tips, the middle, and the bases of the scales, as well as at the cut surface of the stem, and consisted of mycelium taken from agar plates (Pl. 1, C). Similar results were obtained by using infected scales as inoculum. Seventeen tests at 0° C. gave 12 lesions on wounded buds and 2 on unwounded buds. Fifty-one tests at 7° gave 46 lesions on wounded buds and 43 on unwounded buds. Fifty-one tests at 22° gave 48 lesions on wounded buds, also 48 on unwounded buds. Eight tests at 30° gave five lesions on wounded buds, none on unwounded buds. It was difficult to keep the humidity high enough to produce infection at the higher temperatures. Unfortunately, no apparatus was available for making controlled humidity tests.

Except at very low temperatures, inoculation and infection by contact seem limited much more by moisture than by temperature or by the presence of wounds. With adequate moisture, infection took place and was quite independent of wounds, except at very low temperatures. A saturated or nearly saturated atmosphere induces very rank development of mycelium, which, however, as in plate cultures, seldom sporulates unless subjected to a change in humidity. It was necessary to wrap each bud in waxed paper for keeping in moist chambers in the laboratory, in high-temperature incubators, or in incubators cooled by mechanical means which freeze out the air moisture. In low-temperature incubators, cooled by melting ice, wrapping was not necessary.

Experiments with spore suspensions gave negative results except on wounded tissue. Moisture was again a limiting factor, but even more decisively. In early experiments no infection was obtained at any temperature with spore suspensions sprayed or poured on wounded or unwounded scales. Wrapping was entirely inadequate when spore suspensions were used, but the difficulty was finally solved by fogging the chambers containing the buds once every 24 hours during the first three or four days. This was done by spraying water into the chambers with an atomizer. It was a very unsatisfactory method because there was no way of determining just what degree of humidity is essential for spore inoculation; but it duplicated field conditions in that the air

was saturated and water was condensed on the buds at least once a day.

In inoculation tests with spore suspensions made with wounded and unwounded buds at temperatures ranging from −1° C. to 26°, negative results were obtained at −1°, 0°, 0.5°, and 1.5°. Infection resulted occasionally at 5.5°, but only in wounds. The number of infections obtained was greater at 7° than at 5.5°, and still greater at 10°, with a maximum between 15° and 21°. Between 26° and 32° infection was obtained only occasionally. All infection obtained started in artificial or natural wounds (Pl. 1 D). Scales which had been wounded and dipped in spore suspensions developed lesions of 2 to 5 sq. cm. with sporulating mycelium on the surface in nine days at 26°.

The presence or absence of wounds seems, therefore, the most important limiting factor so far as infection by spores is concerned, with moisture, and then temperature next in importance. For infection by mycelium, however, moisture is the main limiting factor, temperature and wounds ranking in the order named.

Infection with spores was not obtainable when the tissues were not wounded. Wounds are abundant and inevitable, however, on both the growing and the harvested buds. There is more or less tearing of the scale tips during growth, for they are curved inward and indented (Pl. 1, A, D) so that tensions arise at the spine and lead to tearing as the scales enlarge. A large wound is made when the bud is cut from the plant in harvesting (Pl. 1, B), and later when buds are crowded into crates for a tight pack there is more tearing of the scales and considerable bruising of the sides as well.

Holding tests made at 7° C. show that the buds of a lot which appear sound at the beginning of a test develop Botrytis decay progressively, and eventually almost all show decay. Possibly this is due to a weakening of the tissues. It is a general observation in the field that *Botrytis* readily attacks aging or weakened parts of plants, such as the outer dying leaves of lettuce or celery. Field observations indicate that the age or vigor of artichoke buds is a factor, inasmuch as the small buds produced at the close of the seasonal productive period or by old plants show greater decay than buds from more vigorous plants, some inspection certificates reporting 4 to 10 per cent decay in large buds and 20 to 30 per cent in small ones. It has also been observed that tissues killed or weakened by overheating or freezing are more readily attacked than healthy ones.

THE DISEASE

PLACE OF ORIGIN, SPREAD, AND DEVELOPMENT

Observations in packing houses indicate that even though some buds with small lesions are packed, by far the greatest number of boxes contain few buds showing any signs of lesions or of the fungus. Field observations indicate that spore contamination in the field is probably the original and chief source of the disease in transit. Here can be found buds and other parts of the plants covered with sporulating mycelium. A much more serious source of infection, however, is the immense amount of plant trash allowed to lie in or adjacent to the fields, which is often covered with an extensive sporulating growth of Botrytis that liberates clouds of spores whenever disturbed. The great quantities of plant trash exist because the bearing plants, which resemble huge thistles, are cut down annually in May and their roots allowed to sprout or sucker. The new tops come into bearing during September and October, and in the fourth or fifth year, when buds begin to be small and hard, the plants are replaced by new shoots.

Examination upon arrival of buds taken out of boxes not previously opened shows that the lesions usually start at the tips of the scales or at the cut stem surface. This indicates that the original infections come from spores that lodge in these places and that secondary infections in transit are primarily by contact. Secondary infection by spores also may take place, since infected scales frequently are covered with sporulating mycelium.

That the bud surfaces carry either spores or mycelium of Botrytis is easily demonstrated by taking buds from packing boxes and exposing them to favorable temperature and moisture conditions. Botrytis rot develops almost invariably. In fact, if the buds were not washed and sterilized, it seemed impossible to have control material come through the experiments, which lasted from several days to several weeks, without developing Botrytis rot. That the contaminations were mostly on the surface is proved by the fact that a few minutes' dipping in corrosive sublimate solution sufficed for sterilization. Thorough washing was also frequently sufficient. Yet occasional development of decay in buds so treated proved that incipient but invisible infections were also present.

It was found that spores or incipient infections may lie dormant for considerable periods if conditions are unfavor-

able, and then begin or resume growth when conditions become favorable. That transit gives ample time for the development of lesions already present and the starting of new ones is indicated by the following data: At 7° C. lesions with a radius of 0.5 to 2 mm. developed in 7 days when wounds were inoculated. Scales inoculated with spore suspensions at 25° developed lesions which had an area of 2 to 5 sq. mm. on the ninth day and were covered with sporulating mycelium. Lesions increased in radius at 10° at the rate of 1.5 mm. every 24 hours; at 15 to 20°, at the rate of 4 to 5 mm.; at 27°, at the rate of only 2 mm.; and at 30° there was no increase. The maintenance of an average temperature of 7° is very good for a refrigerator car. Thus temperature conditions in transit are not unfavorable for the development and spread of the decay.

RELATION TO TEMPERATURE IN THE FIELD AND IN TRANSIT

The optimum temperature for growth of Botrytis lies between 22° and 25° C. The range from this to the maximum of 32° is only 7°, whereas the range to the minimum extends beyond −2°, or more than 27°. Unlike many other fungi, this fungus can make a very appreciable growth and sporulate between 5° and 10°.

The temperatures of the leading artichoke-producing section in California are not unfavorable for the growth of the fungus. The nearest weather reporting station where weather is comparable to that of the Half Moon Bay section is San Francisco, Calif. Here the temperature rarely exceeds the maximum for *Botrytis cinerea* Pers.; the lowest temperature is above its minimum and the mean is below rather than above its optimum.[5] At the annual mean temperature of the region (54.9 °F.), the fungus can enlarge lesions on inoculated buds at the rate of about 4 to 5 mm. per day under favorable moisture conditions.

Abstracts of food products inspection certificates show that the amount and severity of the decay of artichoke buds in transit vary with the temperature. Almost always the decay is heaviest in the two upper layers of car-lot shipments, and in these it is most severe at or near the doors. The following are typical reports: "Ten per cent decay in fourth and fifth layers; none in lower three"; or "Decay in two top layers, 40 to 60 per cent; in lower two, 2 per cent," or "Practically no decay in boxes near bunkers; 30 to 50 per cent in boxes at doors" and "Two top layers, all buds decayed; two bottom layers, slight decay." The direct temperature relation is shown by the temperature report for the last above-mentioned car, in which the temperature of the artichokes was 21° C. at the top of the load in the door, 11° at the bunker, and 4.5° at the bottom of the load at the bunkers.

RELATION TO MOISTURE IN THE FIELD AND IN TRANSIT

The experiments show that while moisture ranks second to wounds as a limiting factor for infection by Botrytis spores, it ranks first as a limiting factor for mycelial growth and infection by mycelium. For sporulation, however, changes in humidity are more important than continuous humidity.

Botrytis rot is generally reported as serious in sections having abundance of foggy weather or in other sections during foggy seasons. Since few regions offer such consistently favorable conditions as the Pacific coast, this probably accounts for the prevalence of Botrytis rot in vegetable shipments from California. The artichoke sections of California lie within the coastal belt of California where fogs are a daily occurrence, so characteristic that the cooperative growers association markets its best brand of artichokes under the trade name "Fog-Kist." During the growing season fogs drift in from the sea every afternoon and are not dissipated until late the next morning, so that vegetation is thoroughly wetted at least once daily.[6]

The artichoke buds are packed in paper-lined boxes which are generally loaded into iced cars. In the early part of the season buds are packed in iced drums. It was found that condensation of water frequently takes place in the buds when the boxes are first placed in the iced cars and that often the buds are moist when they are unloaded in receiving markets. Therefore moisture conditions in transit are also favorable for mycelium development, sporulation, infection, and development of lesions.

[5] U. S. Dept. of Agriculture, Weather Bureau. SUMMARY OF THE CLIMATOLOGICAL DATA FOR THE UNITED STATES, BY SECTIONS. REPRINT OF SECTION 14, CENTRAL AND SOUTHERN CALIFORNIA. 25 p., ILLUS. 1912.

[6] PALMER, A. H. FOG ALONG THE CALIFORNIA COAST. Mo. Weather Rev. 45: 496–499, illus. 1917.

CONTROL OF THE DISEASE

Two lines of control suggest themselves. The first and most effective, if it were possible, is prevention of contamination and, to a lesser extent, of primary infection in the field; the second is prevention of primary spore infection and of secondary contamination and infection in transit.

There was no opportunity for trial of protective sprays or dusts. In general, these have not been very successful in combating diseases caused by *Botrytis* sp. The greatest promise of control in the field lies in sanitary measures, such as the destruction of plant trash. Yet, even though scrupulous sanitation in the artichoke fields would very much lessen the amount of contamination, it would not necessarily eliminate Botrytis entirely, since coastal California weather conditions allow the fungus to grow saprophytically and parasitically on a great range of wild and cultivated plants. Maintenance of the low temperatures which can be obtained in the best refrigerator cars can not completely control the disease because even though low temperatures above the freezing point of the buds retard infection, development, and spread of the disease, they do not control it completely, since the fungus can grow at temperatures low enough to injure and freeze the buds. Moreover, low humidity controls primary spore infection as well as secondary contamination and infection much more than do low temperatures. Infection by spores or by mycelium was not obtainable at any temperatures unless the buds were kept in a humid atmosphere. Unfortunately, equipment was not at hand to determine just what humidity is necessary for spore germination and for infection by spores and mycelium. Control of humidity in refrigerator cars at present is difficult, because the present method of cooling cars by melting ice inevitably leads to a humid atmosphere.

Since these experiments show that even though spore germination and growth can take place at temperatures between −2° C. and 5°, and since infection by spores (the primary method of infection) does not take place at temperatures below 5°, much infection might be prevented if it were possible to get the buds cooled to this temperature immediately after cutting. Once infection has taken place, growth of the fungus and the infection by mycelium, for which wounds are not essential, can take place at temperatures ranging from −2° to 32°. Commercially significant lesions can develop in infected buds at 0° during the 10 to 15 days necessary to get the buds to eastern markets. Nevertheless, maintenance of a temperature of about 5° C. is desirable, as preventing secondary infection by spores, retarding growth of the fungus, and lessening secondary infection by mycelium.

Though some tearing of the tips of scales takes place during growth and a large wound must be made when the buds are cut from the plants, more careful handling of the buds during harvesting and packing could eliminate a great deal of wounding.

Control of the disease is therefore a complex problem, and responsibility for losses in transit or storage is divided among all those who handle the crop. Growers should keep fields and their environs free from plant trash. The packer should avoid packing buds which show discolorations and lesions, since there are indications that buds cut in badly infested fields are more heavily contaminated than those from clean fields. He should not mix badly contaminated and clean buds, should handle them with the greatest care to avoid wounding, and should pack without delay and cool them at once to about 5°. The carrier should provide the most rapid car movement possible, because each additional hour increases the chances of infection with the consequent danger of larger and more numerous lesions. He should also provide a temperature of about 5° while the buds are in transit, and try to prevent saturation of the atmosphere in the car. It is of advantage to the dealer to receive this stock quickly. He should then keep it in fairly dry, refrigerated places if it must be held any length of time.

SUMMARY

1. A rot induced by a Botrytis of the cinerea type causes serious transit losses of shipments of Globe artichoke buds from California. Morphologically, the Botrytis strains isolated from the artichoke (except culture No. 255 from the interior of a frozen stem) seem to be identical with those generally obtained from other vegetables. Cross inoculation tests lend additional weight to the view that a Botrytis of the cinerea type is responsible for practically all Botrytis rot of vegetables.

2. Although disease is of practically no importance in the artichoke fields so far as quality and quantity of marketable buds are concerned, the fields are the original source of con-

tamination, where the fungus attacks vigorous growing plants to some extent, occurring much more frequently on aging plants and on plant trash.

3. The fungus has a wide temperature range, its minimum lying below —2° C., its optimum between 22° and 25° C, and its maximum at about 30° to 33° C. Infection was obtained experimentally throughout the temperature range of the fungus. It is obtained more readily below the optimum than above it. The rate of development of lesions is greater below the optimum than above it.

4. The temperature reactions of the fungus are essentially the same on both agar and bud scales. Mycelium can cause infection without wounds at all temperatures within its temperature range, whereas wounds are necessary for infection by spores.

5. Moisture is the principal limiting factor for both spore and mycelium infection. High humidity followed by a decrease in humidity leads to sporulation. Temperature and especially moisture conditions in the artichoke-producing section of California are favorable for the growth of Botrytis with consequent contamination of the buds.

6. Control involves field sanitation and possibly protective spraying or dusting of the buds; also careful handling to avoid unnecessary wounds in harvesting and packing.

7. Control also involves the maintenance of low humidity and of temperatures of about 5° C. in transit, although even at this temperature complete control is not possible after infection has occurred, because the decay, when once started, progresses at —2°, the freezing temperature of the buds.

8. Responsibility for losses in transit or in storage is not single, but generally rests severally upon growers, packers, shippers, carriers, and storage men, each of whom could contribute some measure of prevention.

THE DEPTH DISTRIBUTION OF THE ROOT-KNOT NEMATODE, HETERODERA RADICICOLA, IN FLORIDA SOILS[1]

By G. H. GODFREY[2]

Pathologist, Office of Cotton, Truck, and Forage Crop Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

In any measure taken to decrease the number of root-knot nematodes in the soil, one of the factors that must be taken into consideration is the depth at which they occur. Whatever treatment may be given, whether chemical, heat, or cultural, must be applied with the idea of reaching the large majority of the parasites, or its effectiveness will be greatly diminished. The experiments described in this paper were designed to give a rough determination of the relative root-knot nematode content of certain soils at different depths, and at different seasons.

LITERATURE

Only the most general information on this subject is available in the nematode literature. Cobb [3] indicates, in regard to soil-inhabiting nematodes in general, that they are likely to be most abundant near the surface and gradually decrease in numbers as the depth increases. Atkinson [4] found root knots on parsnips and tomatoes 15 to 18 inches below the surface of the ground. Larvæ released from such knots would naturally increase very greatly the numbers of nematodes at that depth, for a time at least. Bessey [5] stated, in connection with the overwintering of the organism, that the nematodes probably descend into the lower levels of the soil to avoid the cold. No exact information on this point appears to be available. Frank [6] mentioned the occurrence of galls on deep rooted plants, and discussed the possibility of there being a positive geotaxis due to gravitation.

METHODS

The general methods followed in this experimental work were to collect soil samples at the desired depths from thoroughly infested soils and to grow plants susceptible to root-knot in them under uniform conditions. The number of distinct knots that appeared on the roots within a given period was taken as an index of the number of nemas in the soil, the results being naturally purely relative. It was assumed that a sample producing twice as many knots as another sample had relatively twice as many nemas capable of producing infection. This assumption need not be exactly correct in order that the general results be comparable.

The soil samples were taken in successive layers at depths of 1 to 2 inches, 3 to 4 inches, 5 to 6 inches, 7 to 8 inches, 9 to 10 inches, 11 to 14 inches, 15 to 18 inches, 19 to 22 inches, 23 to 26 inches, 27 to 30 inches, and 31 to 34 inches, respectively. The samples were taken in quadruplicate, each sample being sufficient to fill a 6-inch flower pot.

In collecting the samples every precaution was taken to avoid contaminating any lot with soil from another level. A V-shaped trench was dug to a depth of 34 inches. This is indicated diagrammatically in Figure 1. With a large, flat trowel the 1- to 2-inch sample was taken from the top of the soil at one end of the trench, and the excess soil to that depth lifted and discarded. After taking the sample, the trowel was sterilized by dipping in a boiler of hot water. Then the next layer was removed, the trowel sterilized, and so on until the 11 sets of samples were taken.

[1] Received for publication May 5, 1924—issued January, 1925.

[2] The writer acknowledges credit due to Dr. G. M. Armstrong, now of the Missouri Botanical Garden, St. Louis, Mo., for an active part in this and other nematode problems of the South during the summers of 1920, 1921, and 1922.

[3] COBB, N. A.—NEMATODES AND THEIR RELATIONSHIPS. U. S. Dept. Agr. Yearbook 1914: 457-490, illus. 1915.

[4] ATKINSON, G. F.—NEMATODE ROOT-GALLS. Ala. Agr. Exp. Sta. Bul. 9, 54 p., illus. 1889.

[5] BESSEY, E. A.—ROOT-KNOT AND ITS CONTROL. U. S. Dept. Agr. Bur. Plant Indus. Bul. 217: 36 illus. 1911.

[6] FRANK, B. UEBER DAS WURZELÄLCHEN UND DIE DURCH DASSELBE VERURSACHTEN BESCHÄDIGUNGEN DER PFLANZEN. Landw. Jahrb. 14: 164, illus. 1885.

For the first series of experiments the samples were placed in cloth bags covered with a double wrapping of heavy waxed paper. These were packed in canvas bags, the four samples from each depth together, and the entire lot packed in crates and shipped to Washington. The soil was then placed in 6-inch pots under good growing conditions in the greenhouse, and set to tomato seedlings, three to a pot. In about six weeks, when the earliest infections had reached maturity, and before the production of any secondary infections, the plants were removed, the roots washed, and readings taken on number of root knots per pot.

In the second series of experiments the soil samples were collected direct from the field into 6-inch pots, and the tomato plants grown in them in the propagation houses at the Plant Introduction Garden at Brooksville, Fla.

As stated above, the measure of the relative nematode content of the various soil samples was the number of knots that developed on the roots of the tomato plants grown in them. At first actual counts were made. Based on these counts, a scale was adopted to indicate the relative numbers as follows: 1 to 100 knots, *few;* 100 to 200, *medium;* 200 to 400, *numerous;* 400 to 600, *very numerous;* above 600, *extreme.* The experiments conducted the second year were based not on actual counts but on estimates, recorded in the terms just cited. These estimates were made by Robert Smith, who, because of his long contact with the nematode experimental work, had a very good conception of relative degrees of infestation. It was found by a few counts that such estimates were sufficiently accurate for the purpose.

Unfortunately, some features of these experiments that should ordinarily have received more attention had to be neglected, owing to the pressure of work elsewhere.

RESULTS

In the first series of experiments, made with an extremely sandy loam soil at Brooksville, Fla., collections and plantings were made at intervals of a little more than a month, a total of 10 being conducted from June 28, 1920, to May 27, 1921. The soil samples throughout were shipped to Washington, D. C., and plantings of tomatoes made in the greenhouse. At approximately six weeks after planting, in each case, the plants were lifted, the roots carefully washed, and notes taken on the relative abundance of root-knot infestations.

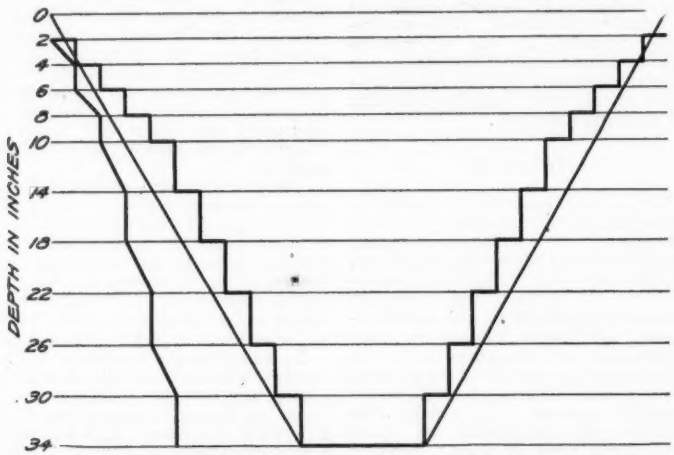


FIG. 1.—A diagram representing the trench dug for collecting soil samples at different depths

The chart (fig. 2) shows graphically, the relative depth distribution of the root-knot nematodes at different dates. In examining this chart it should be borne in mind that differences in infestation may not be significant as seasonal variations. More probably any great difference between one month and the one preceding or following it, for example, is due to distinct differences

in nematode content of the particular spots of ground where the samples were taken. For this series of experiments, these spots were unfortunately in widely different parts of the field, which naturally introduces an uncontrolled difference factor, even though the spots were chosen in each case on account of severe infestation in the crop preceding.

abundant below the tenth inch, and were numerous to the maximum depth examined, 34 inches. In August they were numerous down to 18 inches, and were present in medium or less quantities to the full depth. In September they were extremely abundant in the first 14 inches of soil depth. From December to April the nemas appear to

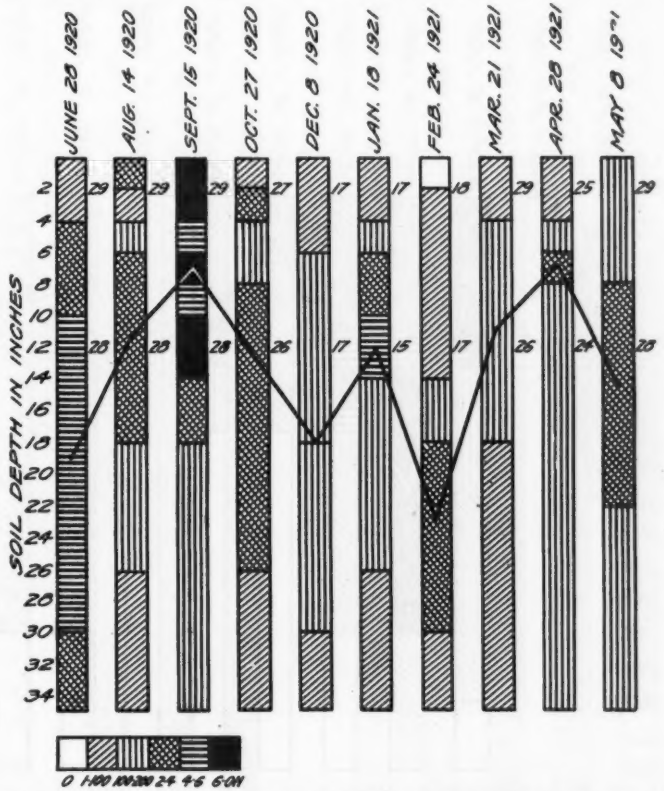


FIG. 2.—Depth distribution of root-knot nematodes in sandy loam soil at Brooksville, Fla., from June 28, 1920, to May 28, 1921. The shading indicates the approximate number of knots that developed on tomato plants grown in the soil samples. The results are the average of four samples. The curve connects central points of regions of maximum infestation. Temperatures in degrees centigrade at 2 and 12 inch depths are also given, these figures being the average for the week preceding date of collection of the sample, at the Plant Introduction Station at Brooksville, Fla.

The differences in nematode content between the different depths in the same spot in the field are within a reasonable degree reliable. The regions of maximum infestation, in the different spots, as determined by samples taken at different times, therefore, are roughly comparable. These regions were, on the whole, several inches beneath the surface. In June the nemas were most

have been less numerous throughout the soil than in the summer months, as might very well be expected. During that period the temperature was below that shown by experiment to be favorable for infection and development, and probably a large proportion of the many free larvæ and eggs may have succumbed to the many unfavorable conditions to which in all probability

they were subjected. Little is known of the actual bionomics of this nematode in its free state within the soil, of its movements and responses to various stimuli, of its relations to other forms of soil life, etc.

The temperatures, recorded on the chart for the 2- and 12-inch depths, show a considerable drop between October and December, but in all probability not enough to have much effect on the vitality of the organisms. The minimum temperature for the winter months at the 2-inch depth, 8° C., occurred on January 16 and again on February 26, 1921. The lowest weekly average at this depth was 17°, and the lowest weekly average at 12 inches was 15°. The curve drawn in the chart between the centers of regions of maximum infestation should indicate any marked seasonal movement, if such occurred. While there appears to be a slight depression in the curve during the winter months, it is probably not sufficiently great to be significant. If similar nematode distribution data and temperature records were secured in a region farther North, where freezing temperatures in the upper layers of soil occur, it is possible that they would show a significant seasonal movement of the nematodes in the soil.

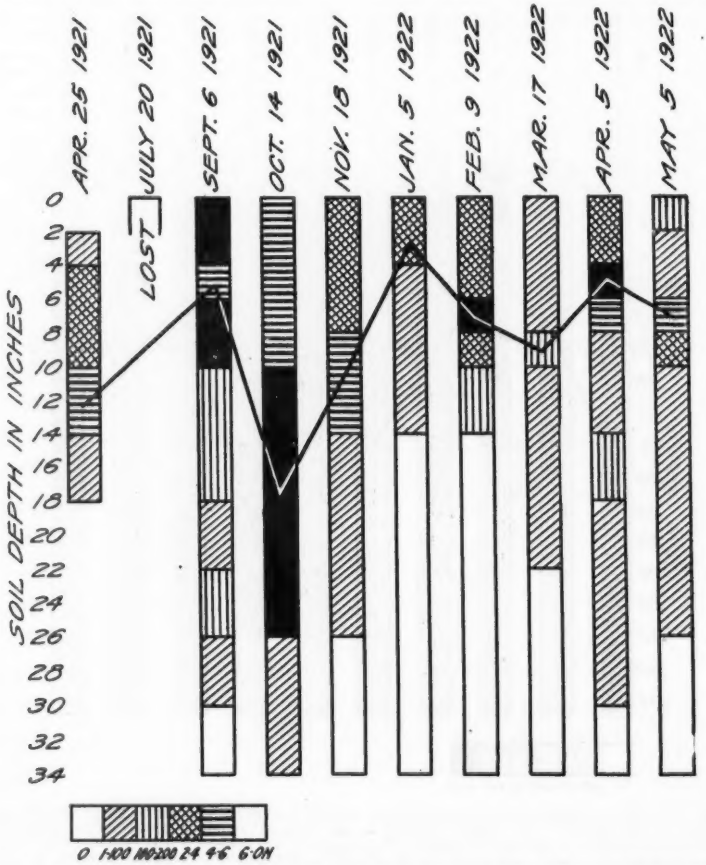


FIG. 3.—Depth distribution of root-knot nematodes in sandy loam or so-called "hammock land," with a heavy clay subsoil, on United States Plant Introduction Garden at Brooksville, Fla., June 28, 1920, to May 28, 1921

Figure 3 gives the experimental data on nematode infestation at various depths on hammock land at the Plant

Introduction Garden at Brooksville, Fla. Here the upper soil is a fertile sandy loam and the subsoil a heavy sticky clay. The subsoil very obviously limits the spread of the nematodes downward. Here, again, extreme variation from one month to another is shown, due no doubt to the actual difference in nematode content of the different areas sampled.

soil, considerable infestation occurs to the maximum depth sampled. The regions of maximum infestation are on the whole, near the surface, though below the ordinary plow depth. In some of the samples, notably those of October, 1921, and March, 1922, very abundant infestation was present deep in the soil.

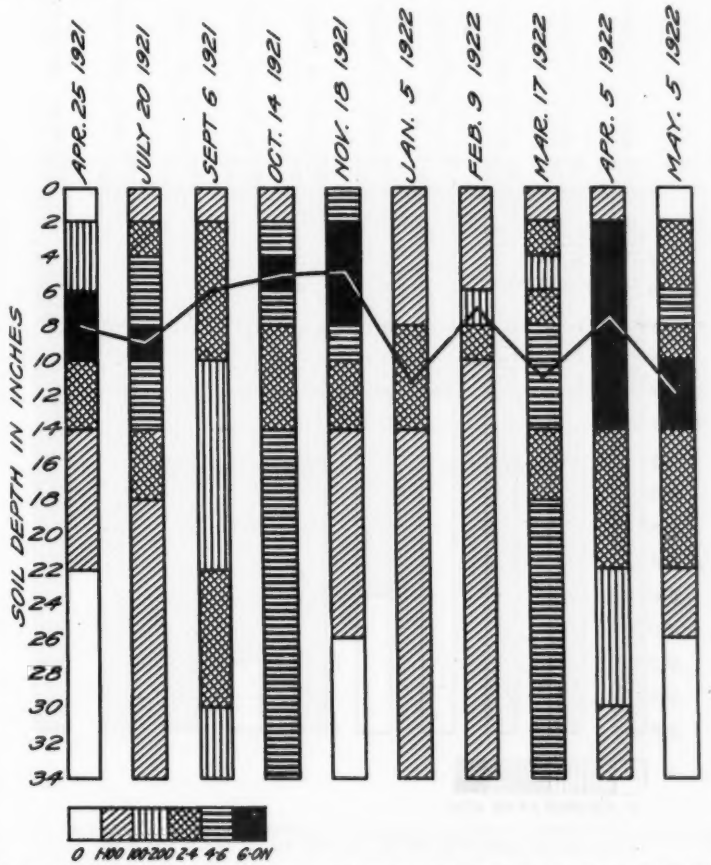


FIG. 4.—Depth distribution of root-knot nematodes in sandy soil at Brooksville, Fla., from April 25, 1921, to May 5, 1922

Figure 4 shows the results of an experiment in which sandy soil from various depths in a root-knot infested field at Brooksville, Fla., was sampled and potted, and planted with tomatoes, at the Plant Introduction Garden. The results in root-knot infestation were recorded by estimates of the relative numbers of knots present on the roots. As in the first year's samples of sandy

Experiment No. 4 was conducted in a similar manner to and simultaneously with experiment No. 3, except that Brooksville hammock land soil was used. The monthly variations in depth of the regions of maximum infestation, as shown in Figure 5, are certainly not significant, as regards seasons, and are obviously due to variations in the soils sampled. This is indicated by com-

paring this chart with Figure 3 in which depths of maximum infestation are distinctly different for the same months.

SUMMARY

Root-knot nematodes are often very numerous to a depth greater than that of the water table; (4) the type of the soil and the subsoil. The influence of such factors may be so great as to overshadow any seeming seasonal change, or at least any that may occur between one month and the next. In winter months a considerable reduction in total root-knot nematode content of the soil appears to be evident.

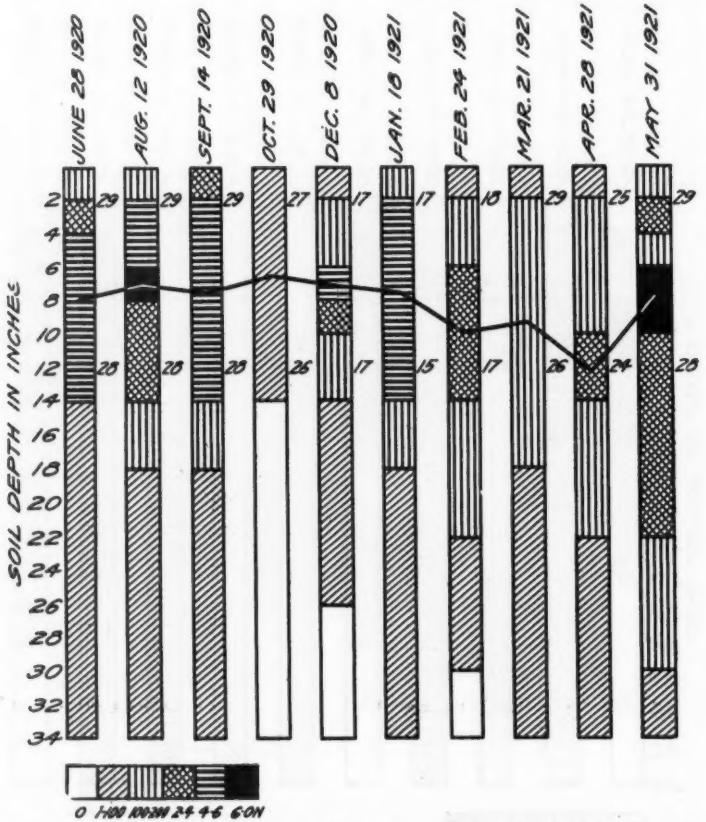


FIG. 5.—Depth distribution of root-knot nematodes on "hammock land" soil at the United States Plant Introduction Garden at Brooksville, Fla., from April 25, 1921, to May 5, 1922

ordinarily reached by a plow. A considerable variation from one spot to another and from one month to another occurs in the depths of the regions of maximum infestation. Many factors no doubt enter into this, such as (1) differences in depth penetration of infested roots; (2) the relationship of time of taking soil samples to a period of heavy rainfall; (3) the height

It is possible that regular seasonal movement does not occur to any great extent unless temperatures approaching freezing occur in the upper layers of soil. The experiments described here indicate that, in order to arrive at conclusive results in this regard, a large number of samples taken from different nematode-infested areas would have to be tested.

ADDITIONAL COPIES

OF THIS PUBLICATION MAY BE PROCURED FROM
THE SUPERINTENDENT OF DOCUMENTS
GOVERNMENT PRINTING OFFICE
WASHINGTON, D. C.
AT
10 CENTS PER COPY
SUBSCRIPTION PRICE, $4.00 PER YEAR (DOMESTIC)
$5.25 PER YEAR (FOREIGN)

▽